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AMELIORATIVE EFFECTS OF GRAVIOLA AND TURMERIC VERSUS TiO_2 NANOPARTICLES INDUCED HEPATOTOXICITY IN RATS

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Abstract

Titanium dioxide (TiO_2) NPs have been broadly used in sunscreens, personal care, paint, pharmaceutical and food products, but it has the ability to generating radical's oxygen species (ROS) including oxidative stress, lipid peroxidation and DNA damage. So, the study evaluated the ameliorative effects of Turmeric and/or Graviola extract against the titanium dioxide nanoparticles (TiO_2) on rats for 1 month. Methods: The study was conducted in 80 Adult male of Swiss Albino rats which divided equally to eight groups: 1st was control: The rats were kept under standard condition, 2nd was Turmeric: The animals received Turmeric 400 mg/kg body weight orally, 3rd was Graviola: The animals received Graviola 400 mg/kg body weight orally, 4th was Turmeric + Graviola: The animals received Turmeric and Graviola, 5th was TiO_2: The animals received 600 mg/kg body weight TiO_2 orally, 6th was TiO_2 + Turmeric: The animals received TiO_2 and Turmeric, 7th was TiO_2 + Graviola: The animals received TiO_2 and Graviola and 8th TiO_2 + Turmeric + Graviola: The animals received TiO_2, Turmeric and Graviola. Results: Administration of TiO_2 in rats induced a significant increase in serum ALT, AST and ALP concentrations and a significant decrease in serum ALB, T.P, B. Cholinesterase and they had higher level of MDA, lower total antioxidant and R.GSH activities. But groups that were administrated with Turmeric and /or Graviola showed improvement. Conclusion: the current results indicate that Turmeric and/or Graviola effectively protect against TiO_2 hepatotoxicity.

***Key words*:** TiO_2 NPs, Graviola, Turmeric, Hepatotoxicity.

Introduction

Nanotechnology recently has taken extensive attention across several areas such as energy, cosmetics, medicine, the environment and health care, drugs, information technology. In the nearby forthcoming, nanotechnology may be capable to resolve various inquiries regarding biological systems that presently load society (Andrews, 2019 and Mostafavi *et al.*, 2019). The lowering of particle sizes were the main target of nanotechnology, leading to enhancement of cellular uptake efficiencies and gives unique physical properties which help in biomedical research (Luzi *et al.*, 2019). Currently, owing to the enormous number of uses of nanomaterials in all fields of life and due to their growing introduction into bio-systems, such as air, water and soil it reasonable to associated problems and threats to humanity in the future (De Matteis and Rinaldi 2018). The size of nanoparticles (NPs) is one of the most important properties of NPs. Hence, the expansive uses of nanotechnology and the great differences that occur among numerous nanomaterials requests the estimation of probable hazards to the human health and environment. The nanoparticles can react with living organisms present in the environment and rise toxic effects, NPs vary in their physicochemical and toxicological properties with regards to the same material on a large scale (Subramaniam *et al.*, 2019).

Titanium dioxide (TiO_2) NP have been broadly used

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in sunscreens, personal care, paint, harmaceutical and food products. TiO_2 NP have presented in two forms, rutile and anatase. The two forms are toxic, the anatase NPs more toxic than rutile NPs and these particles consecutively linked with oxidizing mechanisms of an living organisms, Nano particles capable to producing radical's oxygen species (ROS) as well as oxidative stress, lipid peroxidation and DNA damage (Ibrahim *et al.*, 2015. Su, Li *et al.*, 2018 and Carmo. *et al.*, 2019). Recently, TiO_2 NPs are used in papers, ink, paints, plastics, cosmetics and products of skin care, particularly in the size of 1-100 nm. Furthermore, TiO_2 NPs are marked with high surface area per unit mass, minute size and great activity so NPs pass rapidly through the human body and causing a high risk on the human health (Warheit *et al.*, 2007). The liver is the most target organs of those NPs, because it works on detoxification of the body (Jia *et al.*, 2017). Recently manufactured nanoparticles are orally up taking such as toothpaste, color additive for food as well as capsule and pass easily through the human body in different ways and may disturb the body metabolism (Weir *et al.*, 2012).

Integrated medicine became more important currently, while it presents lots of medicinal plants and plant component selections for treatment of different diseases. The natural medicinal drugs are not only used in treatment but also used as a prophylactic treatment. The medicinal plants are a hope successively for human beings (Inoue *et al.*, 2019). Turmeric (*Curcuma longa*), the bright yellow of the spice rainbow, is a potent remedy that has long been used in the Chinese and Indian protocols of treatment as an anti-inflammatory factor and to treat a lot of disorders, including jaundice, hepatitis flatulence, cystic fibrosis, menstrual distress and rheumatoid arthritis, (Duke, 2007). It also reduces cholesterol level, thus prevents cardiovascular disorders & has an antioxidant effect against liver, kidney diseases and cancer (Qin *et al.*, 2017).

Graviola (*Annona muricata*) is a natural plant was used in the treatment of several diseases. Recently researches demonstrated that the leaf 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 (Kumar and Kumar, 2018). So, the aim of our study is assessment the ameliorative effect of Turmeric and/or Graviola against TiO_2 hepatotoxicity in rats.

Materials and Methods

Experimental animals

80 male of Swiss albino rats, their weights 170±20.0 g, brought from the National Research Centre (NRC) animal house, Cairo University, Egypt. Under typical environmental conditions the animals were kept on 12 hour light/dark cycle under a constant temperature of (25±1)°C, water and nutrition were permitted all the time.

Experimental design

All animals were divided into eight equal groups as the following: Group (1), control (The animals received balanced diet. Group (2), the animals were given orally 400 mg/kg BW Turmeric once daily (García-Niño *et al.*, 2013), Group (3), the animals received 400 mg/kg BW Graviola orally once daily (Usunomena, 2014), Group (4), the animals received 400 mg/kg BW Turmeric and 400 mg/kg BW Graviola orally once, Group (5), the animals received 600 mg/kg BW TiO_2 NPs (1/20 LD 50) orally once daily (Ibrahim *et al.*, 2018), Group (6), the animals received 600 mg/kg BW TiO2 NPs (1/20 LD 50) and 400 mg/kg BW Turmeric orally once, Group (7), the animals received 600 mg/kg BW TiO_2 NPs (1/20 LD 50) and 400 mg/kg BW Graviola orally once daily, Group (8), the animals received 600 mg/kg BW TiO_2 NPs (1/20 LD 50), 400 mg/kg BW Turmeric and 400 mg/kg BW Graviola orally once daily. The time period of the experiment for all groups was 30 days.

Preparation of TiO_2 nanoparticle suspensions

TiO_2 NPs brought from Loba Chemie, Mumbai city, India. The nanoparticles were UV sterilizer and stock suspensions were saved in Buffer Saline of Phosphate (PBS), shaken during 2 minutes, sonicated for 30 min and stored in the dark at 4°C until use and shaking well before gavaging (Joo *et al.*, 2013).

Chemicals (Reagent)

Turmeric (Curcumin): Rapid release gelatinous Capsules, (400 mg/Capsule) was purchased from Vitamin World, Inc. Ronkonkoma, NY 11779 U.S.A. and kept at a 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 a 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 tools were cleaned with cleanser and acid and washed with distilled water.

Weight and Dissection of animals

Animal weights were recorded daily and finally, the animals were starved through the night, sacrificed according to the Ethics Committee of the National Research Centre. The animals immolated by cervical dislocation and samples were gathered from each animal into two tubes both with and without anticoagulants. The liver, were dissected out. Blood was taken from all animals in each group and centrifuged at 3000 rpm for 10 minutes. Plasma and Serum samples were preserved at 0°C until biochemical analysis for aminotransferase (AST), alanine aminotransferase (ALT), total protein concentrations and Butyryl Cholinesterase concentration in the same day (Sapan *et al.*, 1999 and Giacobini, 2001). Livers were removed, cleared from adhering connective tissue. a Part of liver was kept for histopathological study in 10% fixative formalin, the remained part for to the analysis of antioxidant and oxidative stress indicators.

Liver homogenate Preparation

Liver tissue was smoothed and homogenized for 10 sec., then mixed with 1 to 9 of phosphate buffer, in an ice bath. The resultant homogenates were taken for centrifugation at 19,000 RPM at 4°C for 30 minutes and transfer the supernatants into clean tubes and kept at-80°C until using (Diederich and Michalke, 2011).

Antioxidant and Lipid Peroxidation Analysis

The levels of malondialdehyde (MDA) in hepatic tissues were determined according to the method of Satoh, 1978, the level of R.GSH in hepatic tissues according to the method of Beutler *et al*., 1963.

Histopathologic investigation

The animals were starved before being sacrificed and the liver was fixed in 10% neutral buffer formalin for different investigations.

Statistical Analysis

Data were expressed by frequency, Mean+SD and percentage. One-way (ANOVA) was used to test significance and p-value<0.05 was considered statistically significant.

Results

Toxicity symptoms

Behavioral changes were observed in the animal groups gavaged orally with Titanium dioxide nanoparticles such as inactiveness, loss of appetite, nerves. Other slight toxicity symptoms were observed such as gray and white faces, brittleness of skin hair. Most of these symptoms disappear from Titanium dioxide groups which co-treated with Turmeric and/or Graviola.

Change in body weight

In table 1, different animal group's weights demonstrated as mean ± standard deviation of initial and final body weights of the experimental animals. Experimental control (control rats, rats treated with turmeric and/or Graviola or both of them) showed non-significant increase in the body weight gain as normal control so the control groups indicate to these groups while a significance decreasing in body weight of Titanium dioxide group only when compared with control and others titanium treated groups so there weren't body weights gain but a significant increasing in body weight of Titanium treated groups (Ti_2O+Turmeric, Ti_2O+Graviola and Ti_2O+Turmeric+Graviola) treated group when compared with control group.

Liver functions

The ALT and AST concentrations were highly significant increasing in Titanium dioxide injected group while a mild significant increasing in Titanium co-treated groups (Titanium+Turmeric), (Titanium+Graviola) groups, but in (Titanium+Turmeric+Graviola) group, the ALT and AST concentrations were not altered when compared with control group, while in the experimental control groups treated with turmeric or Graviola or both of them, there weren't change in the ALT and AST concentrations as control. Also, total protein (T.P), Albumin (ALB) and

Table 1: The animal weight changes in different experimental animals.

Groups	1st weight (g)	Final weight (g)	changed weight (g)
Control	134.2±4.08	140.8±5.71	6.6
Turmeric	136±4.89	142±6.81	6.0
Graviola	137±6.74	142.4±8.01	5.4
Turmeric+Graviola	136.4±4.5	144.2±5.71	7.8
Titanium	134.6±4.72	131±5.87	-3.6*
Ti +Turmeric	140.2±5.54	148.4±10.87	8.2*
Ti+Graviola	138.2±4.49	146.6±3.57	8.4*
Ti+Turmeric+Graviola	140.2±4.08	150±4.35	9.8*

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

Table 2: The liver functions (ALT, AST, ALB, Total protein and Globulin and Albumin) in different experimental animals.

Groups	ALT (U/L)	AST (U/L)	Total protein (g/dL)	ALB (g/dl)	Globulin (g/dL)
Control	42.06±5.66	68.2±11.25	6.53±0.2	4.42±0.15	2.1±0.34
Turmeric	50.48±7.47	86.57±15.64	6.56±0.28	4.436±0.135	2.13±0.2
Graviola	53.68±6.78	70.8±17.07	6.78±0.28	4.408±0.229	2.37±0.15
Turmeric + Graviola	41.24±4.03	67.44±16.13	6.67±0.22	4.48±0.286	2.19±0.38
Titanium dioxide (Ti_2O)	116.12±18.05**	444.26±41.09**	4.97±0.4*	3.25±0.227**	1.72±0.21*
Ti_2O + Turmeric	54.46±2.10*	196.98±19.89**	6.18±0.65	3.894±0.189*	2.29±0.57
Ti_2O + Graviola	70.02±10.09*	271.4±40.15**	6.3±0.55	3.593±0.234*	2.77±0.43
Ti_2O +Turmeric + Graviola	49.36±5.83	156.78±15.83**	6.54±0.16	3.824±0.27*	2.72±0.36

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

Globulin (G) levels were highly significant reduction in Titanium dioxide group, while in titanium co-treated groups (Turmeric or Graviola and Turmeric and Graviola) showed a significant lowering in treated rats compared to the control one as presented in table 2.

Alkaline phosphatase (ALP) concentration in titanium group was a highly significant increase when compared with the control group, but in Titanium co-treated groups (Turmeric, Graviola and Turmeric+Graviola), the concentration of the ALP was significantly reduced when compared with the group and slightly increased when compared with the control group. Butyryl cholinesterase (P.ChE) concentration in titanium group and Ti_2O+ Graviola group B.ChE concentrations were highly significant decrease when compared with the control group, while in others Titanium dioxide co-treated groups (Turmeric and Turmeric+Graviola) the concentrations of P.ChE were significantly decreased when compared with the control group. Also, the total antioxidants were significant decreasing in the group while the best elevation was recorded in one group of the experimental control groups (Turmeric+Graviola) when compared with the normal control group. In case of titanium treated groups there were no changes if compared to the Experimental control.

Reduced Glutathione was highly significant decreasing in the Ti_2O group while the R.GSH was significant decreasing in The Ti_2O+Turmeric and Ti_2O+Graviola groups. But in case of Titanium+Turmeric +Graviola group, there were no differences when compared with control one. The MDA was highly significant Increasing in Ti_2O group while the MDA was significant increasing in Ti_2O+Turmeric and Ti_2O+ Graviola groups. But In case of titanium+Turmeric+ Graviola group, there was marked improvement approximately near to the results of the control group as shown as in table 3.

Histopathological observations

There were no histological differences between Experimental control groups (Turmeric, Graviola and Turmeric+Graviola) and control group, so the term control is applicable to both groups. The functional unit of the liver is the hepatic lobule which is formed of radiating strands of cells around the central vein. The liver cell strands are alternating with narrow blood sinusoids. The hepatic sinusoids are narrow spaces of blood with single layer of fenestrated endothelial cells and Kupffer cells (phagocytic cells). The hepatic cells are polyhedral in shape

Table 3: Alk. Phosphatase, Butyryl Cholinesterase, the antioxidants and oxidative stress Concentrations in different experimental animals.

Groups	Alk. Phosphatase (U/L)	Butyryl Cholinesterase (U/L)	Total Antioxidants mM/L	R.GSH mg/dL	MDA nmol/mL
Control	184.84±21.05	552.22±53.34	5.32±0.08	5.06±0.72	1.49±0.25
Turmeric	171.94±17.72	531.46±85.38	5.42±0.09	4.81±0.60	1.55±0.42
Graviola	196.42±98.13	504.54±65.45	5.35±0.08	4.91±0.62	1.78±0.51
Turmeric + Graviola	180.12±41.31	568.64±88.15	5.57±0.03*	5.25±0.60	1.24±0.19
Titanium dioxide(Ti_2O)	435.52±68.37**	196.5±56.3**	4.36±0.71*	2.35±0.42**	5.63±0.84**
Ti_2O + Turmeric	197.59±37.97	374.04±37.62*	5.37±0.16	3.64±0.59*	2.57±0.57*
Ti_2O + Graviola	262.1±36.06*	263.7±39.19**	5.36±0.20	3.03±0.75*	2.52±0.75*
Ti_2O+Turmeric +Graviola	233.89±44.11	410.32±56.95*	5.35±0.15	4.27±0.43	1.98±0.30*

The data were expressed as Mean±SD, *=P<0.05 and **=P<0.0.

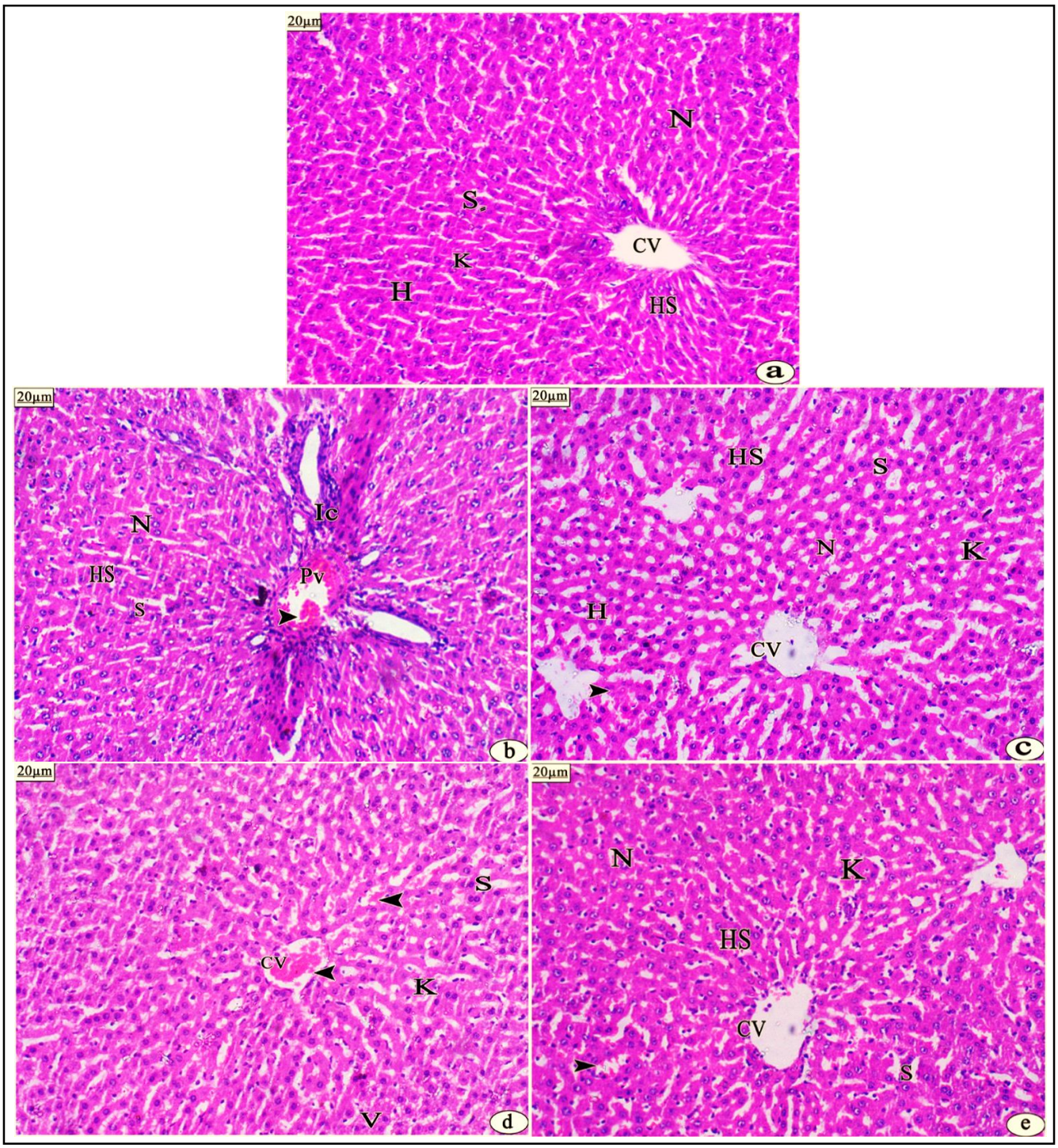

Fig. 1: (a): Liver section of control Rats: showing a central vein (CV) in the middle. Within each lobule, hepatocyte cells (H) are arranged into hepatic stands (HS) running radiantly from the central vein and are separated by adjacent sinusoids (S). With normal nuclei (N) and normal Kupffer cells are observed (K). (b): Liver section of TiO_2 NPs Rats: showing congestion (►) in a portal vein (P), sever degeneration in liver cells with massive invasion of mononuclear leucocytic inflammatory cells (arrows head), especially around portal vein (PV). Accumulation of mononuclear cells inflammatory cells (IC), binucleated hepatic cells (N) and pyknotic nuclei were present. (c): Liver section of TiO_2 NPs Rats treated with Turmeric: showing some blood dilated blood sinusoids (s), hepatocyte cells (H) are good arranged into hepatic stands (HS). (d): Liver section of TiO_2 NPs Rats treated with Graviola. (1 e): Liver section of TiO_2 NPs Rats treated with both of Turmeric and Graviola: showing little dilated sinusoids (►), normal central vein (*CV*) in the middle with in each lobule, hepatocyte cells (H) are arranged into hepatic stands (HS) running radiantly from the central vein and are separated by adjacent sinusoids (S) and normal Kupffer cells are observed (K).

and a granular cytoplasm. Each cell is with centrally located nucleus and one or two nucleoli as shown in fig. 1a.

Microscopic examination of liver sections of the TiO_2 NPs group showed different signs of injury in where most of them were hypertrophied, faintly stained and vacuolated cytoplasm. In addition, some degenerated hepatocytes could be seen in different areas of the liver tissue as shown in fig. 1b, dilated congested blood sinusoids, some degenerated binucleated cells were observed with faint eosinophilic cytoplasm and chromatolytic nuclei. The liver architecture showed several damages as some hepatic strands were dissociated. This is probably due to dilatation of the central veins and hepatic sinusoids. Particularly, leukocytes inflammatory cells were observed all over the liver tissue.

Microscopic examination of hepatic sections taken from rats of titanium dioxide group co-treated Turmeric (TiO_2 NPs+Turmeric) showed that the Turmeric enhancement the most abnormal injuries which caused by Titanium NPs except some little congestion in dilated sinusoids and some hepatocytes showed slight signs of injury such as swelling of their nuclei as shown in fig. 1c. Also, the Graviola recovered for the most abnormalities were caused by TiO_2 NPs except some little swollen in hepatocytes with vascular degenerated cytoplasm and some congestion in central vein as shown in fig. 1d.

Microscopic examination of liver sections taken from rats of TiO_2 NPs+Turmeric+Graviola group showed that the Turmeric and Graviola improved most abnormalities which caused by Titanium NPs except some little dilated sinusoids and vacuolated cytoplasm. While the hepatocytes were with normal appearance with little effects of TiO_2 NPs on the liver tissue than observed in other TiO_2 NPs groups as shown in fig. 1e.

Discussion

Nanotechnology is the latest technology, which spread highly in several basic sciences, biotechnology, agriculture, food resources and medicine (Luzi *et al.*, 2019). The Pollution of Nanoparticles is considered as a new problem (Gupta, 2019). Metallic dioxid nanoparticles marked with high Stability in the environment and food chain so causes maintenance of their poisonousness (Kumari *et al.*, 2019). Nanoparticles have high surface area due to its small size. they have a perfect capability to passage through the tissues. The blood and lymphatic circulatory systems can transport Nano-TiO_2 into several organs in the body (Hong and Zhang, 2016 and Hong *et al.*, 2016). The toxicity of Nanoparticles due to oxidative stress, interfering with membrane structure, binding protein or DNA, producing active oxygen (ROS) and cell death or apoptosis (Unfried *et al.*, 2007). In the current study, toxicity of titanium dioxide nanoparticles was investigated due to their recurrent application in industries.

In the current study animals that received TiO_2 NPs by gavaged revealed some toxicity signs and clinical signs such as reduction food and water consumption, diarrhea, nervous and gray to white faece were observed. Most of these symptoms disappear after treatment with Turmeric and/or Graviola. Body weight changes are generally a serious factor in toxicological studies (Machtay *et al.*, 2008). The saving of a sufficient body weight is a main cause of the survival of mammals**.** The current results detected the TiO_2 NPs treated group showed remarkable significance decreasing in body weight if compared with control groups. in contrast, a significant increasing in body weight in titanium treated groups (TiO_2+Turmeric, TiO_2+Graviola and TiO_2+ Turmeric+ Graviola). (Shakeel *et al.*, 2018) stated that animals injected subcutaneous with titanium dioxide with dose 150 mg/kg TiO_2 NPs showed reduction in body weights and increase death rate where two animals died during the last week of the experiment. Fartkhooni *et al.,* (Fartkhooni *et al.*, 2016) showed the effects of intraperitoneal injections (IP) of TiO_2 NPs (30, 50, 70 mg/kg) for 21 days, (11 times) on adult Male albino rats. They found that body weight of rats in all groups before and after treatment didn't show any significant difference, may be due to use small doses of TiO_2 NPs (30,50,70 mg/kg) for 21 days, an alternate day (11 times) but in our experiment we use 1/20 LD_{50} of TiO_2 NPs (600 mg/kg) daily for 30 day, or due to the kind of animal or different physical and chemical properties of nanoparticles or the route of administration of nanoparticles as oral, respiratory, dermal and number of injections (Shubayev *et al.*, 2009).

The oral giving out of 600 mg/kg BW of TiO_2 NPs (69 nm) for 30 successive days induced hepatotoxicity marked by significant increases in liver enzymes (ALT, AST and ALP) and significant decrease in Alb, Globulin, Total protein, B. Cholinesterase, Liu *et al.*, (2010) tested biochemical parameters in mouse livers, finding that when the concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), pseudocholinesterase (PChE), lactate dehydrogenase (LDH), triglycerides, total protein and albumin all increase, inflammatory cascades are activated and lead to liver injury characterized by liver cell apoptosis. The ALT and AST levels were elevated in the TiO_2-treated group, which showed a loss of functional probity of the liver cells and membrane leakage (Sha *et al.*, 2014). The current results showed that TiO_2 NPs treated group had high significant increased ($P \leq 0.001$) on serum liver

function enzymes ALT, AST and ALP compared with control groups (Ma *et al.*, 2009). In addition, the results of experiments in which various doses of nano-TiO_2 in mice were injected intraperitoneally showed marked increases in the levels of ALT and AST of liver (Bruno *et al.*, 2014). According to the current results we found that oral administration of 400 mg/kg BW of Turmeric for 30 consecutive days protects the hepatotoxicity caused by TiO_2 NPs via decreasing liver Functions (ALT, AST and ALP) and increasing in Alb, Globulin, Total protein, B. Cholinesterase concentrations.

Khorsandi *et al.,* (2016), studied the protective effect of curcumin against hepatotoxicity of ZnO NPs, hefindsd out ZnO induced a significant increase in plasma AST (2.8-fold), ALT (2.7-fold) and ALP (1.97-fold) activity in comparison to the control group ($p<0.01$). Hence, the hepatoprotective effect of Cur may be due to its antioxidant action. In the current study, we observed that oral administration of 400 mg/kg BW of Graviola extract for 30 consecutive days decreased the TiO_2 NPs-induced elevated ALT, ALP and AST as well as increased total protein, albumin, globulin and B. Cholinesterase level in the treated groups. This suggests the maintenance of structural integrity of the hepatocytic cell membrane or regeneration of damage liver cells by the extract (38). From the results of biochemical parameters carried out on the animals, treatment with *A. muricata* leaf restored the liver enzyme parameters. A serum ALP level also related to the status and function of hepatic cells. Thus, lowering effect of enzyme content in serum is a definite indication of hepatoprotective action of the drug (18). *A. muricata* leaf flavonoids are known to be antioxidants, free radical scavengers and anti-lipoperoxidant leading to hepatoprotection. The mechanism by which *A. muricata* exerts protection against TiO_2 NPs toxicity in the liver may be due to the anti-oxidative and acetogeninic effect of the plant extract. The protective effects may be the result of stabilization of plasma membrane, thereby preserving the structural integrity of the cell as well as the repair of hepatic tissue damage (Oyedepo, 2014).

Enzymes such as reduced glutathione, glutathione peroxidase, total antioxidants, superoxide dismutase and catalase are the main system that opposes oxidation. The current results showed that the activity of these enzymes showed a marked depletion ($P\leq0.001$) when rats gavaged TiO_2 NPs compared with control groups. In addition to working as a direct free-radical scavenger, GSH also functions as a substrate for GPx. 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 elevations of MDA the indicative for lipid peroxidation (LPO) and significant reductions for R.GSH concentration as well as antioxidant enzyme activity (Total antioxidants).

Schanen *et al*., (2009) stated that due to Tio_2 NPs photosensitivity, nano-TiO_2 can become a substance that generates reactive oxygen species (ROS) in the body, resulting in radical metabolic imbalances and abnormal increases in ROS. Excessive ROS produce toxicity, resulting in the formation of biofilms and macromolecular substances that induce lipid peroxidation damage. When ROS is harmful, organisms use a variety of enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) to clear ROS. $O_2^{\bullet-}$ is converted to H_2O_2 and O_2 by SOD, whereas CAT and GSH-Px can eliminate H_2O_2 by converting it to H_2O and O_2. Accordingly, SOD, CAT and GSH-Px keep ROS at low levels, effectively protecting cells from the toxic effects. Excessive production of ROS breaks the balance of the liver oxidant/antioxidant system, resulting in lipid peroxidation and liver cell apoptosis. In the current study, the decreased R.GSH concentration, Total antioxidants and GPX enzyme activities and elevated MDA levels indicated that the generation of oxidative stress upon TiO_2 NPs intoxication (Natarajan *et al.*, 2015). The current study revealed that titanium dioxide co-treated groups (Titanium+ Turmeric or Titanium+ Graviola or Titanium+ Turmeric+ Graviola) showed marked improvement occurred in antioxidant enzymes and lipid peroxidation. However, the activities of the antioxidant enzymes Reduced glutathione, glutathione and total antioxidants were slightly increased when treated with turmeric. In addition, the intake of Turmeric significantly elevates the level of oxidative enzymes in turmeric-treated groups.

According to the obtained results we found that oral administration of TiO_2 NPs (69 nm) induced marked hepatotoxicity marked by histopathological changes in the liver, where TiO_2 NPs causing liver cell necrosis in the lobules around the central vein, severe degeneration in liver cells with a massive invasion of mononuclear leukocytes inflammatory cells, especially around the portal vein (PV), dilated sinusoids and cytoplasmic vacuolation. Confirming that nano-TiO_2 is toxic to the liver, the powerful penetrative abilities of nano-TiO_2 allow it to enter into various organs of the body. Upon deposition in the internal organs, nano-TiO_2 generates toxicity, causing organ damage. Ti^{4+} accumulation increased in each organ, including the brain and especially the liver, kidney and spleen. Administered nano-TiO_2 primarily accumulates in the livers of rats, with an accumulation of 69% after administration for 5 min and 80% after administration for

15 min (Huggins and Froehlich 1966). After mice were orally treated with nano-TiO_2 for 30 days, the liver showed congestion and other diseases, as well as decreased levels of leukocytes and T cells, glutamate aminotransferase and AST, which are all related to liver function. These results indicate that nano-TiO_2 impairs the immunity of the liver and alters liver function (Li *et al.*, 2010).

On the other hand, in relation to the lack of toxicity of nanoparticles can mention to studies that suggest by passing time and after one month that the density of nanoparticles is reduced in the liver, spleen, kidneys and lungs, also, the distinctive changes have not been reported in the enzyme level, which have been measured in blood serum and no toxicity in the organs which confirms the nanoparticles of titanium dioxide can be safety used in low doses (Han *et al.*, 2009).

Turmeric's anti-inflammatory properties may be attributed to its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid and neutrophil function during inflammatory states (Hanai and Sugimoto, 2009). In the current study, titanium groups treated with 400mg/kg body weight of the Graviola extract showed no significant histological changes. So the Graviola extract has significant anti-hepatotoxic, activity against TiO_2 NPs induced hepatotoxicity in albino Wistar rats. In the histopathological studies, it was clearly found that the typical architecture of liver tissue was observed in control and Graviola group rats with a central vein (CV) from which chords of hepatocytes were radiating. In TiO_2 NPs Group necrosis, degeneration, disarrangement of the hepatocytes along with inflammation in the sinusoids, dilated blood vessels and sinusoidal spaces flooded with inflammatory cells and RBC was observed. High protection was observed with 400mg/kg body weight dose of *A. muricata* leaf extract. There was no significant necrosis although signs of inflammation were present. Central and portal veins were also found to be normal with a normal sinusoidal space. That mean Graviola (*A. muricata*) showed mild to moderate improvement in toxicity. Treatment with *A. muricata* leaf extract restored the hepatic architecture and protected the liver tissue from cytoplasmic degenerative changes, by preventing the toxic chemical reaction, oxidative stress, lipid peroxidation, molecular changes in the liver tissues, micro and macro vesicular fatty changes ultimately leading to necrosis (Puri *et al.*, 2019).

Conclusion

The present study concluded that the current results indicate that TiO_2 produces more toxic effects on liver and considered as a major caustic effective agent. It was recommended that the using of Turmeric and/or Graviola should be avoided from hepatotoxicity induced by TiO_2.

Significance Statement

This study confirmed that exposure to TiO_2 at doses of 600 mg/kg BW, produces more toxic effects on liver and considered as a major sharp effective agent against animal health when it received for 30 days. The study contributes to the effective monitoring of studies Turmeric and/or Graviola consumption and avoiding the hepatotoxicity induced by TiO_2.

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