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Original Article

THE POTENTIAL MODULATORY EFFECT OF RUTIN ON TITANIUM DIOXIDE NANOPARTICLES-INDUCED RENAL INJURY IN MALE MICE

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ABSTRACT

Objective: This study aimed to investigate the possible protective effect of rutin in management of TiO_2NPs-induced renal injury in mice.

Methods: Forty male Swiss albino mice were randomly divided into four groups (n=10). Group (I) served as a control group, group (II) received 100 mg/kg body weight (b. wt) of rutin (orally), group (III) received 70 mg/kg b. wt of TiO_2NPs,injected intraperitoneally (i. p.), Group (IV) received 70 mg/kg b. wt of TiO_2NPs plus 100 mg/kg b. wt of rutin; for 14 successive days. The renal toxicity was determined through evaluating the renal function biomarkers (serum creatinine, urea, and uric acid) and the levels of malondialdehyde (MDA), reduced glutathion (GSH), nuclear factor kappa B (NF-κB), tumor necrosis factor-α (TNF)-α, B-cell lymphoma (BCL)-2 and caspase-3 in renal tissues.

Results: Administration of TiO_2NPs plus rutin prevented the deleterious effect of TiO_2NPs on mice kidneys through improving the renal functions, and alleviating the increase in MDA, NF-κB, TNF-α, and caspase-3 levels, as well as the decrease in GSH andBCL-2 levels, in renal tissues.

Conclusion: Taken together, these findings suggested that rutin plays a role in alleviating TiO_2NPs-induced oxidative stress, inflammation, and apoptosis, and exerts renal protective effects.

Keywords: Apoptosis, Renal functions, Rutin, TiO_2NPs, Toxicity

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INTRODUCTION

According to the National Nanotechnology Initiative of America, titanium dioxide nanoparticles (TiO_2NPs) are considered one of the maximum noticeably synthetic global nanomaterial's [1, 2]. The TiO2NPs are repeatedly used in food industry, manufacturing of vitamin, dietary supplements, and crop manufacturing [1, 2]. In addition, TiO_2NPs are implemented broadly in other certain fields as medicine, paints, surface coatings, and environmental decontamination regarding air, soil, and water [1]. Although TiO_2 is taken into consideration to be a safe material, worries had been raised its potential adverse health effects in humans and animals [3]. As a result of its poisonous potential, TiO_2 has been categorized by way of the International Agency for Research on Cancer as "likely carcinogenic to humans" within breathing [4]. When inhaled nanoparticles are efficiently deposited into lung cell, translocation *via* epithelial and endothelial cells among the blood and lymph circulation may be reached sensitive target sites such as bone marrow, lymph nodes, spleen, heart, liver, and kidneys [5]. A major route of human exposure to TiO_2NPs is through food intake, where TiO_2NPs are used widely as food-coloring agents, and as a pharmaceutical additive [1]. The photocatalytic properties of TiO2NPs caused many toxic effects in the lungs [6], liver [7] spleen [8], brain [9, 10], reproductive system [11] and heart [12] of mice.

The kidneys are one of the most sensitive organs to toxic substances in the body because of its high blood flow and its ability to accumulate wastes [13]. For instance, TiO_2NPs accumulated in the kidneys of rainbow trout [14]. It also increased the blood urea nitrogen and creatinine levels in blood of mice, due to it caused renal injury and toxicity [15, 16]. The intragastric administration of TiO_2NPs induced accumulation of reactive oxygen species (ROS) such as O_{2^-} and H_2O_2 in mouse kidneys resulted in oxidative damage of biological macromolecules, including lipids, proteins, and DNA, coupled with inflammatory response and apoptosis in the renal tubules [17].

Plant flavonoids possess a remarkable spectrum of pharmalogical activities like antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, antimutagenic, antineoplastic, as well as neuroprotective properties [18]. Among the flavonoids, rutin is a glycone of quercetin having a flavonol structure. Inside the intestine of animals, rutin is converted to quercetin by microflora and absorbed by intestinal cells [19]. Rutin is consumed in the daily diet, such as buckwheat, tomato, onions, orange, lemon, apple, and tea [20]. Arjumand *et al.* [21] observed that pretreatment of rats with rutin inhibits cisplatin-induced renal toxicity. This effect was accompanied by a marked reduction in the inflammatory and apoptotic pathway. Therefore, the main objective of the current research was to investigate the renoprotective effect of rutin on oxidative stress, inflammation, and apoptosis in TiO_2NPs-induced renal toxicity in mice.

MATERIALS AND METHODS

Experimental animals

Forty male Swiss albino mice (*Mus musculus*), aging 9-12 w old and weighting 25-30 g, were obtained from the Helwan Breeding Farm, Cairo, Egypt. Animals were breed and maintained in an air-conditioned animal house, subjected to a 12: 12 dlight/darkness, and allowed limitless access to chow and water. The animals were acclimated for a period of two weeks before the starting of the experiments. The experiment was carried out in accordance with the laboratory animals guidelines approved by the Research Ethical Committee of Medical Research of National Research Centre, Giza, Egypt (registeration number: 13/165).

Chemicals

The TiO_2NPs (>100 nm particle size) were purchased from and characterized by Sigma-Aldrich Corp (St. Louis, MO USA). The TiO_2-NPs were suspended in a double distilled water and ultrasonicated using ultrasonic cleaner CD-4831 (MEDISERVIS Czuczor s. r. o, Nové Zámky, Slovakia) at Ac 220-240 v, 50 HZ, 170 W for 30 min. Rutin hydrate powder ($C_{27}H_{30}O_{10}$. xH_2O, purity ≥ 94% HPLC) were purchased from Sigma-Aldrich Corp.

Experimental design

The animals were randomly divided into four groups (n=10), as follows: Group (I) received 0.5 ml distilled water and served as a

control group, group (II) received 100 mg/kg body weight (b. wt) of rutin was taken orally, group (III) received 70 mg/kg b. wt of TiO_2NPs injected intraperitoneally (i. p), and group (IV) received 70 mg/kg b. wt of TiO_2NPs+100 mg/kg b. wt of rutin daily for 14 consecutive days.

Samples' collection

At the end of the experiments animals were sacrificed, Blood samples were collected and serum samples were obtained in clean and dry test tubes; by leaving to clot for 10 min and then centrifuged at 3000 rpm for 20 min and kept at −20 °C until further analysis. Also, kidney was dissected out, washed with phosphate-buffered saline (PBS), and stored at −20 °C. The tissues were immediately rinsed with cold Tris-KCl buffer (0.15 mol, pH 7.4, Sigma-Aldrich Corp) and homogenized in Tris-KCl buffer (1.0 g tissue in 10 ml Tris-KCl buffer) using Teflon homogenizer (Glas-Col LLC, Terr Haute, IN, USA). Thereafter the homogenate was centrifuged at 10000 x*g* and 4 °C for 30 min, and the supernatant was used for the biochemical assay.

Biochemical assays

Serum Creatinine level was estimated by creatinine assay kit purchased from BioAssay Systems (San Jose, CA, USA) according to the manufacturer's instruction. Serum Urea and Uric acid levels were estimated by urea and uric acid assay kits purchased from Sigma-Aldrich Corp according to the manufacturer's instructions. Malondialdhyde (MDA) level was assayed by MDA assay kit purchased from LsBio (Seattle, WA, USA) according to the manufacturer's instructions. Reduced glutathione (GSH) was assayed by GSH assay kit purchased from Shanghai BlueGene Biotech CO., LTD (Shanghai, China) according to the manufacturer's instructions. Tumor necrosis factor (TNF)-α level was assayed by MyBioSource mouse TNF-α ELISA assay kit (San Diego, CA, USA) according to the manufacturer's instructions. The levels of NF-κB, B-cell lymphoma (BCL)-2, and caspase-3 were assayed by MyBioSource mouse ELISA assay kits for NF-κB, BCL-2, and Caspase-3, respectively, according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean±standard error (n=10). The data were analyzed using statistical package for social science software (SPSS 20, SPSS Inc. Chicago, IL, USA) for windows. Differences among experimental groups were determined by one-way analysis of variance (ANOVA), followed by Duncan's [22] method of multiple comparisons. Value of $P<0.05$ among groups was considered statistically significant.

RESULTS

The results showed that the group treated with rutin alone did not significantly change at all biochemical parameters as compared with the control group (fig. 1-4). On the other hand, TiO_2NPs induced a significant increase ($P<0.05$) in serum creatinine, urea, and uric acid levels,as compared with the control group, indicating an impairment in the renal functions (fig. 1). However, the group treated with TiO_2NPs plus rutin showed a significant improvement ($P<0.05$) in the renal functions, as compared with the TiO_2NPs alone treated group (fig. 1).

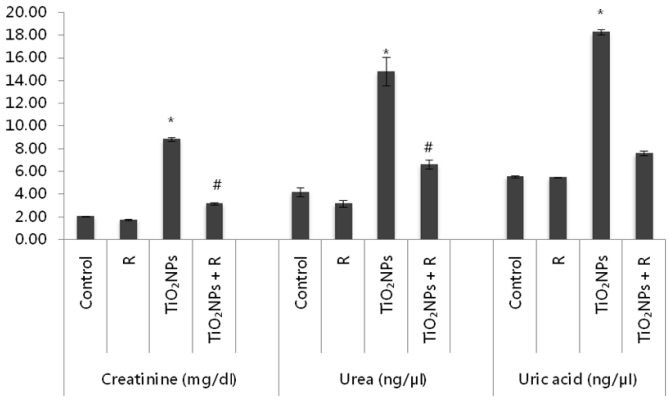

Fig. 1: Effect of rutin (R) on creatinine, urea, and uric acid levels in serum of titanium dioxide nanoparticles (TiO_2NPs)-treated mice. Data are expressed as mean±standard error (n=10). *($P<0.05$): significant difference from the control group, #($P<0.05$): significant difference from the TiO_2NPs treated group

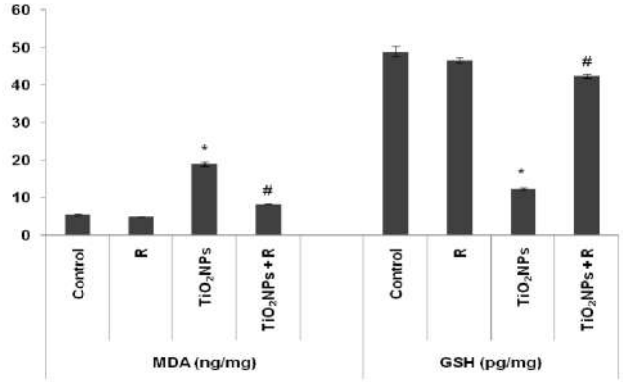

Fig. 2: Effect of rutin (R) on malondialdehyde (MDA) and reduced glutathione (GSH) levels, in renal homogenate of titanium dioxide nanoparticles (TiO_2NPs)-treated mice. Data are expressed as mean±standard error (n=10). *$P<0.05$: significant difference from the control group, #$P<0.05$: significant difference from the TiO_2NPs treated group

The MDA level in renal tissues of TiO_2NPs-treated group elevated significantly (P<0.05) when compared with the control group (fig. 2). Whereas, the co-administrated of TiO_2NPs with rutin induced a significant decrease (P<0.05) in lipid peroxidation in renal tissues in comparison to those received the TiO_2NPs alone (fig. 2). A significant depletion (P<0.05) in the renal GSH level was seen in the TiO_2NPs-treated group, as compared with the control group (fig. 2). Meanwhile, the renal GSH level in the TiO_2NPs plus rutin treated group increased significantly (P<0.05), when compared with that of TiO_2NPs alone treated group (fig. 2).

A significant increase (P<0.05) in the renal NF-κB and TNF-α level was noticed in the TiO_2NPs-treated group, as compared with the control group, indicating an enhancement of the inflammatory response (fig. 3). However, the treatment with TiO_2NPs plus rutin caused a significant decline (P<0.05) in the levels of these inflammatory markers in comparison with the TiO_2NPs alone treated group (fig. 3).

TiO_2NPs led to a significant decrease (P<0.05) in the renal BCL-2 level and a significant increase (P<0.05) in the caspase-3 level as compared with the control group, indicating an enhancement of apoptosis (fig. 4). In this regard, co-administration of TiO_2NPs with rutin significantly alleviated (P<0.05) the apoptotic effect of TiO_2NPs on renal tissues (fig. 4).

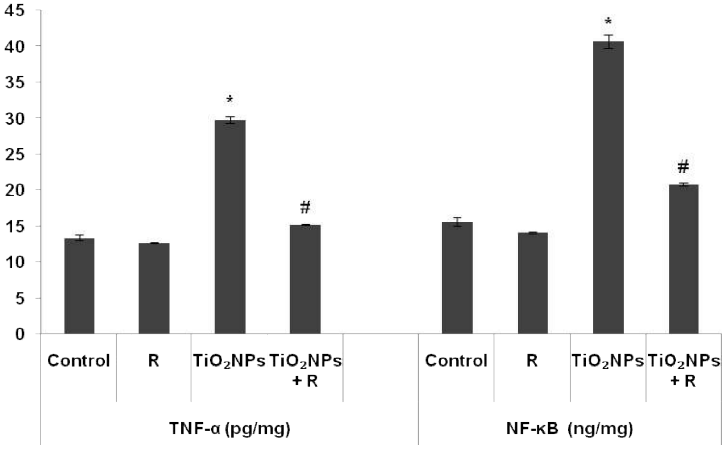

Fig. 3: Effect of rutin (R) on nuclear factor kappa B (NF-κB) and tumor necrosis factor-α (TNF-α) levels in renal homogenate of titanium dioxide nanoparticles (TiO_2NPs)-treated mice. Data are expressed as mean±standard error (n=10). *P<0.05: significant difference from the control group, #P<0.05: significant difference from the TiO_2NPs treated group

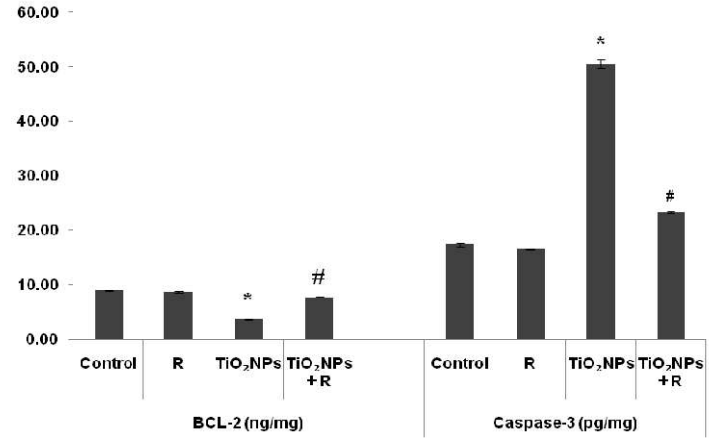

Fig. 4: Effect of rutin (R) on B-cell lymphoma (BCL)-2 and caspase-3 levels in renal homogenate of titanium dioxide nanoparticles (TiO_2NPs)-treated mice. Data are expressed as mean±standard error (n=10). *P<0.05: significant difference from the control group, #P<0.05: significant difference from the TiO_2NPs treated group

DISCUSSION

The current study indicated that animals exposed to the intraperitoneal injection of 70 mg/kg of TiO_2NPs for 14 d caused renal dysfunction in mice as established by significant increase levels of serum creatinine, urea and uric acid. It is may be due to kidney injury which cause released these biomarkers into blood stream. Other scientists also reported the impairment of kidney functions with extreme renal inflammation in animals exposed to different doses of TiO_2NPs [23, 24]. In addition, the quantity of blood urea and uric acid was extensive increased after i. p injection of TiO_2NPs in male rats [25]. Moreover, Borm *et al.* [26] stated that when nanoparticles enter the blood, they may be removed by different mechanisms depending on the path of absorption and the character of their surface, the most common way to remove the nanoparticles is through the kidneys.

In the current study, rutin improved the kidney function of TiO_2NPs-treated mice. Sadeghnia *et al.* [27] also reported that rutin modulated the hexachlorobutadiene (HCBD)-induced renal

abnormalities. Rutin has been shown to alleviate the chronic kidney disease induced by the chemotherapeutic agents like doxorubicin [28]. In addition, rutin significantly attenuated the renal damage caused by gentamicin through alleviating the oxidative stress and inflammation, as well as apoptosis, in experimental animals [29].

TiO_2NPs-terated mice showed a significant rise in the level of renal MDA. Elevated MDA level in the kidney indicated the availability of oxidative stress and lipid peroxidation that have been generated by TiO_2NP [30]. The mechanism of the generation of the oxidative stress after nanoparticles treatment is not clear, but Singh *et al.* [31] suggested that it may be related to the large surface area of the particles together. As noted in the present results, rutin reduced significantly the TiO_2NPs-induced renal lipid peroxidation. This finding may be attributed to the anti-lipoperoxidant effect and the free-radical scavenge activity of rutin [32, 33]. The lipoperoxidant effect of rutin may be also resulted from its ability to scavenge free radicals and chelate metals catalysts [34, 35]. In addition, rutin supplementation markedly reduced the levels of ROS and MDA in kidneys of CCl_4-treated mice [36].

The current results revealed that the group treated with TiO_2NPs showed a significant decrease in the GSH contents. Gui *et al.* [17] also reported that as the dose of TiO_2NPs increased the GSH level in the kidneys decreased. Similar result was obtained in the human epidermal cells after exposure to TiO_2NPs, which was attributed to the over production of ROS in response to TiO_2NPs toxicity [37]. The depletion of GSH in the kidney of TiO_2NPs-treated rats in the present study was associated with the increases in MDA, suggesting its role in preventing lipid peroxidation.

The present data recorded a significant increase in GSH content in the TiO_2NPs-treated animals by rutin. Arjumand *et al.* [21] also found that the rutin increased significantly the renal GSH content in the cisplatin-treated animals. Amelioration of renal GSH level in TiO_2NPs intoxicated rats by quercetin (the active metabolite of rutin) may relate to the antioxidant potential effect of quercetin [24].

Progression of renal disease in TiO_2NP-treated mice is largely driven by inflammation and oxidative stress [17]. TiO_2NPs led to nephric inflammation, cell necrosis, and dysfunction, as a result of activating the NF-κB that enhanced the expression of proinflammatory cytokines such as TNF-α [23, 24]. These findings are in agreement with the present study, where TiO_2NPs increased the NF-κB and TNF-α levels in the mice renal tissues. In the present study both NF-κB and TNF-α levels were decreased by rutin in TiO_2NPs-treated group. NF-κB is a necessary transcriptional mediator for production of the proinflammatory cytokines that plays a pivotal role in the injury of tubular epithelial cells through generating ROS [38]. Qu *et al.* [39] established that a protecting effect of rutin on vancomycin-caused renal damage was through reducing the NF-κB expression and TNF-α generation to the control levels. In addition, rutin alleviated cisplatin-induced renal injury through its inhibitory effect on NF-κB and TNF-α pathway mediated inflammation [21]. Moreover, Yoo *et al.* [40] suggested that rutin might be a candidate therapeutic agent for treatment of different severe vascular inflammatory diseases.

In the present work, the TiO_2NPs-treated group showed a significant reduction in the renal BCL-2 (the anti-apoptotic family protein) level, while a significant increase in the renal caspase-3 activity, indicating the enhancement of renal apoptosis. However, rutin alleviated the renal apoptosis that induced by the TiO_2NPs. Inflammation and apoptosis in renal tubules following exposure to TiO_2NPs may be due to excess of ROS production [17]. Ramkumar *et al.* [41] mentioned that the exposure of human cervical adenocarcinoma HeLa cell line to the TiO_2 nanofibers resulted in a down regulation of BCL-2, possibly by enhancing the oxidative stress. This in turn prompted the leakage for the mitochondrial membrane and the translocation of cytochrome c from mitochondria to the cytosol that activated caspase-3 and promoted apoptosis of the HeLa cell line [41]. Arjumand *et al.* [21] noted that rutin down regulated caspase-3 expression in the renal tissues of cisplatin-treated rats in a dose dependent manner. This result suggested a possible role of rutin in diminishing the signal generated either *via* integral membrane death receptor proteins or *via* mitochondrial cytochrome c release pathway, and finally reducing the activation of effector caspase-3, thereby attenuating the apoptotic death and disruption concerning renal tubular cells induced by cisplatin [21]. In addition, rutin supplementation attenuated significantly vancomycin-induced renal apoptosis through decreasing the activity of caspase-3 and caspase-9 [39]. Moreover, Ma *et al.* [36] reported that different doses of rutin supplementation decreased the release of cytochrome c and the activity of caspase-3 in kidneys of CCl_4-treated mouse.

CONCLUSION

In conclusion, the results of the present work demonstrated the toxic impact of TiO_2NPs on renal tissue of mice. In addition, rutin showed potent ameliorative effects against renal toxicity, inflammation, and apoptosis, as well as and improved renal functions, in the TiO_2NPs-tretaed mice. Moreover, rutin was safe and did not show any undesirable effect on normal mice with the used dose.

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AUTHORS CONTRIBUTIONS

Heba abdul rauf: Being a single author, I carried out a compilation of literature, an inscription of a manuscript, read-through, revising, and improving the standard of the manuscript. No other co-author contributed to this work.

CONFLICT OF INTERESTS

No conflicts of interest

REFERENCES

1. Skocaj M, Filipic M, Petkovic J, Novak S. Titanium dioxide in our everyday life; is it safe? Radiol Oncol 2011;45:227-47.
2. Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol 2012;46:2242-50.
3. Elsaesser A, Howard CV. Toxicology of nanoparticles. Adv Drug Delivery Rev 2012;64:129-37.
4. IARC Working Group. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, (Volume 93, Carbon Black, Titanium Dioxide and Talc). IARC, Lyon, France; 2006.
5. Liu H, Ma L, Zhao J, Liu j, Jan J, Ruan J, *et al.* Biochemical toxicity of nano-anatase TiO_2 particales in mice. Biol Trac Elem Res 2009;129:170-80.
6. Li B, Ze Y, Su Q, Zhang T, Sang XZ, Cui YL, *et al.* Molecular mechanisms of nanosized titanium dioxide-induced pulmonary injury in mice. PLoS One 2013;8:e55563.
7. Cui Y, Liu H, Ze Y, Zhang ZL, Hu YY, Cheng Z, *et al.* Gene expression in liver injury caused by long-term exposure to titanium dioxide nanoparticles in mice. Toxicol Sci 2012;128:171-85.
8. Sang X, Li B, Ze Y, Hong J, Ze X, Gui SX, *et al.* Toxicological mechanisms of nanosized titanium dioxide-induced spleen injury in mice after repeated peroral application. J Agric Food Chem 2013;61:5590-9.
9. Ze Y, Zheng L, Zhao X, Gui SX, Sang XZ, Su JJ, *et al.* Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice. Chemosphere 2013;92:1183-9.
10. Mohamed HA. The neurotoxicity of titanium dioxide nanoparticles in mice. RJPBCS 2019;6:168-76.
11. Zhao X, Ze Y, Gao G, Sang XZ, Li B, Gui SX, *et al.* Nanosized TiO_2-induced reproductive system dysfunction and its mechanism in female mice. PLoS One 2013;8:e59378.
12. Sheng L, Wang X, Sang X, Ze YG, Zhao XY, Liu D, *et al.* Cardiac oxidative damage in mice following exposure to nanoparticlate titanium dioxide. J Biomed Mater Res A 2013;101:3238-46.
13. L'Azou B, Henge Napoli MH, Minaro L, Mirto H, Barrouillet MP, Cambar J. Effects of cadmium and uranium on some *in vitro* renal targets. Cell Biol Toxicol 2002;18:329-40.
14. Scown TM, van Aerle R, Johnston BD, Cumberland S, Lead JR, Richard O, *et al.* High doses of intravenously administered titanium dioxide nanoparticles accumulate in the kidneys of

rainbow trout but with no observable impairment of renal function. Toxicol Sci 2009;109:372-80.

15. Wang J, Zhou G, Chen C, Yu HW, Wang TC, Ma YM, *et al.* Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett 2007;168:176-85.
16. Gui S, Sang X, Zheng L. Intragastric exposure to titanium dioxide nanoparticles induced nephrotoxicity in mice, assessed by physiological and gene expression modifications. Part Fibre Toxicol 2013;10:4.
17. Gui S, Li B, Zhao X, Ze YG, Zhao XY, Sheng L, *et al.* Renal injury and Nrf_2 modulation in mouse kidney following chronic exposure to TiO_2 nanoparticles. J Agric Food Chem 2013; 61:8959-68.
18. Harborne JB. Plant phenolics. In: Encyclopedia of Plant Physiology (Bell, E. A. and Charlwood, B. V. eds) Springer Verlag, Berlin; 1986. p. 329-95.
19. Manach C, Morand C, Demigne C, Texier O, Regerat F, Remesy C. Bioavailability of rutin and quercetin in rats. FEBS Lett 1997;409:12-6.
20. Ghorbani A. Mechanisms of antidiabetic effects of flavonoid rutin. Biomed Pharmacother 2017;96:305-12.
21. Arjumand W, Seth A, Sultana S. Rutin attenuates cisplatin induced renal inflammation and apoptosis by reducing NFκB, TNF-α and caspase-3 expression in wistar rats. Food Chem Toxicol 2011;49:2013-21.
22. Duncan BD. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957;13:359-64.
23. Gui S, Zhang Z, Zheng L, Cui Y, Liu X, Li N, et al. Molecular mechanism of kidney injury of mice caused by exposure to titanium dioxide nanoparticles. J Hazard Mater 2011;195:365-70.
24. Al-Rasheed NM, Faddah LM, Mohamed AM, Abdel Baky NA, Al-Rasheed NM, Mohammad RA. Potential impact of quercetin and idebenone against immuno-inflammatory and oxidative renal damage induced in rats by titanium dioxide nanoparticles toxicity. J Oleo Sci 2013;62:961-71.
25. Fartkhooni FM, Noori A, Mohammadi A. Effects of titanium dioxide nanoparticles toxicity on the kidney of male rats. Int J Life Sci 2016;10:65-9.
26. Borm PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, *et al.* The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol 2006;3:11.
27. Sadeghnia HR, Yousefsani BS, Rashidfar M, Boroushaki MT, Asadpour E, Ghorbani A. Protective effect of rutin on hexachlorobutadiene-induced Nephrotoxicity. Ren Fail 2013;35:1151-5.
28. Peng CC, Hsieh CL, Ker YB, Wang HY, Chen KC, Peng RY. Selected nutraceutic screening by therapeutic effects on doxorubicin-induced chronic kidney disease. Mol Nutr Food Res 2012;56:1541-58.
29. Kandemir FM, Ozkaraca M, Yildirim BA, Hanedan B, Kirbas A, Kilic K, *et al.* Rutin attenuates gentamicin-induced renal damage by reducing oxidative stress, inflammation, apoptosis, and autophagy in rats. Ren Fail 2015;37:518-25.
30. Liang G, Pu Y, Yin L, Liu R, Ye B. Influence of different sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress. J Toxicol Environ Health A 2009;72:740-5.
31. Singh S, Shi T, Duffin R, Albrecht C, van Berlo D, Höhr D, *et al.* Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO_2:role of the specific surface area and of surface methylation of the particles. Toxicol Appl Pharmacol 2007;222:141-51.
32. Negre Salvayre A, Affany A, Hariton C. Additional antilipoperoxidant activities of alpha-tocopherol and ascorbic acid on membrane-like systems are potentiated by rutin. Pharmacology 1991;42:262-72.
33. Oomah BD, Mazza G. Flavonoids and antioxidative activities in buckwheat. J Agric Food Chem 1996;44:1746-50.
34. Kamalakkannan N, Prince PSM. Rutin improves the antioxidant status in streptozotocin-induced diabetic rat tissues. Mol Cell Biochem 2006;293:211-9.
35. Ferrali M, Signofrini C, Caciotti B, Sugherini L, Ciccoli D, Giachetti D, *et al.* Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity. FEBS Lett 1997;416:123-39.
36. Ma JQ, Liu CM, Yang W. Protective effect of rutin against carbon tetrachloride-induced oxidative stress, inflammation and apoptosis in mouse kidney associated with the ceramide, MAPKs, p53 and calpain activities. Chem Biol Interact 2018;286:26-33.
37. Shukla RK, Sharma V, Pandey AK, Singh S, Sultan S, Dhawana A. ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. Toxicology *In Vitro* 2011;25:231-41.
38. Cantaluppi V, Quercia AD, Dellepiane S, Ferrario S, Camussi G, Biancone L. Interaction between systemic inflammation and renal tubular epithelial cells. Nephrol Dial Transplant 2014;29:2004-11.
39. Qu S, Dai C, Lang F, Hu L, Tang Q, Wang H, *et al.* Rutin attenuates vancomycin-induced nephrotoxicity by ameliorating oxidative stress, apoptosis, and inflammation in rats. Antimicrob Agents Chemother 2019;63:1545-18.
40. Yoo H, Ku SK, Baek YD, Bae J. Anti-inflammatory effects of rutin on HMGB1-induced inflammatory responses *in vitro* and *in vivo*. Inflamm Res 2014;63:197-206.
41. Ramkumar KM, Manjula C, Gnanakumar G, Kanjwal MA, Sekar TV, Paulmurugan R, *et al.* Oxidative stress-mediated cytotoxicity and apoptosis induction by TiO_2 nanofibers in HeLa cells. Eur J Pharm Biopharm 2012;81:324-33.