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Modulation efficiency of clove oil nano-emulsion against genotoxic, oxidative stress, and histological injuries induced via titanium dioxide nanoparticles in mice

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Titanium dioxide nanoparticles (TiO₂-NPs) have found wide applications in medical and industrial fields. However, the toxic effect of various tissues is still under study. In this study, we evaluated the toxic effect of TiO₂-NP on stomach, liver, and kidney tissues and the amelioration effect of clove oil nanoemulsion (CLV-NE) against DNA damage, oxidative stress, pathological changes, and the apoptotic effect of TiO₂-NPs. Four groups of male mice were subjected to oral treatment for five consecutive days including, the control group, the group treated with TiO₂-NPs (50 mg/kg), the group treated with (CLV-NE) (5% of the MTD), and the group treated with TiO₂-NPs plus CLV-NE. The results revealed that the treatment with TiO₂-NPs significantly caused DNA damage in the liver, stomach, and kidney tissues due to increased ROS as indicated by the reduction of the antioxidant activity of SOD and Gpx and increased MDA level. Further, abnormal histological signs and apoptotic effect confirmed by the significant elevation of p53 expression were reported after TiO₂-NPs administration. The present data reported a significant improvement in the previous parameters after treatment with CLV-NE. These results showed the collaborative effect of the oils and the extra role of nanoemulsion in enhancing antioxidant effectiveness that enhances its disperse-ability and further promotes its controlled release. One could conclude that CLV-NE is safe and can be used as a powerful antioxidative agent to assess the toxic effects of the acute use of TiO₂-NPs.

Keywords Titanium dioxide nanoparticle, Clove oil nanoemulsion, Titanium dioxide nanoparticles toxicity, Oxidative stress

The versatility of TiO₂-NPs in many care products and commercial applications increases the possibility of human exposure to TiO₂-NP during the manufacturing and processing of the nanomaterial and via certain consumers' goods (eg, sunscreens, enamels, varnishes, pigments for paint)¹. The small size of TO₂-NPs enables their uptake by cells and their transcytosis across the epithelium and endothelium cells, and their passage to the circulatory system to reach many target tissues, accordingly, their accumulation induced many inflammatory and toxic effects^{2,3}. They increase the phagocytic capability of macrophages which provoke membrane and ultra-structure destruction and dysfunction of the liver and kidney⁴⁻⁶. This may highlight the possibility of changing the architecture of many vital organs like the kidney, liver, and lungs due to exposure to TiO₂-NPs^{7,8}. Moreover, TiO₂-NPs can induce oxidative stress which raises the induction of lipid peroxidation that promotes genotoxicity and apoptosis within liver tissue^{3,9,10}.

Cloves (*Syzygium aromaticum* L.) belongs to the *Myrtaceae* family, from which clove essential oil (CEO) is extracted from their flower buds and leaves. CEO contains many phenolic compounds, and eugenol is the major

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compound (85–95%)¹¹. Eugenol has proven its efficiency as an antioxidant agent in many researches^{12,13}. Further, eugenol revealed an anticancer activity against the human adenocarcinoma cell line in the lung¹⁴. Recently, many other biological effects of eugenol like anti-proliferative, anti-inflammatory, anti-microbial, and DNA damage protective effects have been reported^{15–20}.

Nanoemulsion is composed mostly of an essential oil emulsion system composed of an average size droplet size of 200 nm. These oils mostly suffer from solubility differences between droplets size due to different radius curvature which is called Ostwald ripening (OR)²¹. Thus, nanoemulsion is mainly used to overcome the solubility problem and to increase the surface area of the oil. Therefore, the present study aimed to assess the attenuation ability of CLV-NE against genotoxic, cytotoxic, histopathological, and biochemical effects detected after the administration of TiO₂-NPs.

Material and methods

Animals

Thirty 6-week-old Swiss webster mice weighing 25–30 g were obtained from the National Organization for Drug Control and Research (Giza Egypt) and kept in the animal house of the Zoology Department, Faculty of Science Cairo University under standard housing conditions and given food and water ad libitum. Mice were fed completely nutritional diet called Mazuri's vegetarian rat and mouse diet manufactured by Land O' Lakes Inc Company (Arden Hills, Minnesota, USA).

Ethical consideration

This study was reported according to ARRIVE guidelines and all experiments of this study were and conducted according to the standard international guidelines for the care and use of laboratory animals and were approved by October University for Modern Sciences and Arts, Faculty of biotechnology Committee (Egypt). Animals handling and experimentations were also conducted in accordance with the Guidelines of the National Institutes of Health (NIH) regarding the care and use of animals for experimental procedures.

Chemicals

Clove nano-emulsion was purchased from Naqaa Company for nanotechnology (Giza Egypt) with a concentration of 12–15%, biochemical kits were obtained from Bio-diagnostic Company (Giza, Egypt), while all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) with high analytical grade.

Characterization of TiO₂-NPs

A mixture of rutile and anatase-shaped TiO₂-NPs were purchased from Sigma-Aldrich (St. Louis, MO, USA) with purity of 99.5% and CAS number 13463-67-7. Prior to administration, the unscented white powders of TiO₂-NPs were sonicated in deionized distilled water using an ultrasonic homogenizer (Model 150VT) and have been well characterized using transmission electron microscope, X-ray diffraction and Zeta Sizer²².

Characterization of the clove oil nanoemulsion

The appearance and morphology of CLV-NE were studied as described by El-Shamy²³, using transmission electron microscopy (TEM). One drop of CLV-NE was placed on a carbon laminated copper grid, then spread and stained using phosphotungstic acid 1.5%.

Determination of maximum tolerated dose (MTD)

Acute toxicity assay was done to detect the maximum tolerated dose of CLV-NE using OECD guidelines (Guideline 2001). Five male mice were orally given clove nano-emulsion at a dose level of 2000 mg/kg according to the main test of OECD/OCDE. All mice were then carefully observed for 24 h after CEO nano-emulsion administration for any sign of toxicity, morphological behavior, and mortality. The studied doses of clove nano-emulsion were estimated as 5% of the safety dose obtained from the OECD test.

Experimental design

Twenty male mice were randomly divided into four groups, five mice per group; mice of the negative control group were orally given deionized distilled water, while mice of the other three remaining groups were orally administered CLV-NE at a dose of 5% of the MTD or/and TiO₂-NPs at a dose of 50 mg/kg²² for five consecutive days. After 24 h of the last administration, mice of the four groups were sacrificed and dissected to obtain stomach, liver, and kidney tissues. Part of these tissues was kept in 10% formalin for histological studies and other part was kept at –80 °C for molecular and biochemical examination.

Molecular studies

Laddered DNA fragmentation assay

The integrity of genomic DNA in stomach tissues of four groups was studied using Laddered DNA fragmentation assay²⁴; small part of stomach tissues was gently homogenized in TE lysis buffer and incubated with RNase A at 37 °C for cells lysis. Proteinase K was then added, and all samples were incubated at 50 °C overnight. Whole genomic DNA was extracted and precipitated by cold absolute ethanol. The extracted genomic DNA was electrophoresed on 1% agarose gel at 70 V, visualized and photographed.

Expression of p53 gene

The whole gastric RNA was extracted using the GeneJET RNA Purification Kit (Thermo scientific, USA). The purity and concentration of the extracted gastric RNA were detected by a Nanodrop device. The extracted gastric total RNA was then reverse transcribed into complementary DNA (cDNA) using the Revert Aid First Strand cDNA Synthesis Kit (Thermo scientific, USA). Finally, quantitative RT-PCR reaction was conducted to amplify gastric p53 gene through the 7500 Fast system (Applied Biosystem 7500, Clinilab, Egypt) using 2 × SYBR Green Master Mix (Thermo Scientific, USA) and the primers sequences listed in Table 1²⁵. The mRNA expression level of the p53 gene was standardized against expression of β actin housekeeping gene. The comparative Ct ($\Delta\Delta Ct$) method was used to quantify the expression levels of p53 gene and the results were expressed as mean ± S.D.

Biochemical analysis

Parts of Stomach, liver and kidney tissues were homogenized in 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA, then centrifuged at 4000 rpm for 15 min in a cooling centrifuge (Sigma, D-37520 Osterode. Am Harz., Germany, Model 2–16 K), and the supernatant was pipetted into plastic tubes for determination of SOD and GpX as antioxidants according to the method of²⁶ and²⁷, respectively. According to²⁸ method, MDA level was determined using Bio diagnostic kit following the instruction' protocol where the absorbance of the resultant pink product can be measured at wave length 534 nm.

Histological studies

For histopathological studies, fresh portions of the stomach, liver and kidney tissues were fixed in 10% buffered formalin immediately before being embedded in paraffin wax, then they were sectioned into 5 μm thickness using microtome for staining using hematoxylin and eosin. For investigation and analysis, the stained sections were photographed under light microscope.

Statistical analysis

The Social Science Statistical Package (SPSS 23) was used to analyze the results obtained. A one-way analysis of variance (ANOVA) was performed to compare the effects of different treatments on the parameters studied. Duncan's test was performed to compare study groups. Data were expressed as mean ± standard deviation (SD).

Results

Characterization of clove oil nanoemulsion

Assessment of the external morphology of the CLV nanoemulsion by TEM²³ revealed that they had a uniform size distribution measured at 218.1 ± 2.27 nm and a spherical shape (Fig. 1).

MTD and tested dose of CLV-NE

Surveillance of mice orally given CLV-NE at the 2000 mg/kg dose level revealed that all mice were still healthy and no signs of toxicity were observed during the first 48 h of CLV-NE administration until the end of the 14-days monitoring period. Thus, the MTD and the half lethality dose (LD50) of CLV-NE was considered above 2000 mg/

Gene	Strand	Sequence
P53	Forward	5'-TACTCTCCTCCCTCAATAAG-3'
	Reverse	5'- ACCATCGGAGCAGCCCTCAT-3'
β-actin	Forward	5'-TCACCCACACTG TGCCCATCT ACG A-3'
	Reverse	5'-GGATGCCACAGATTCCATACCCA-3'

Table 1. Sequences of the Primers used in qRT-PCR.

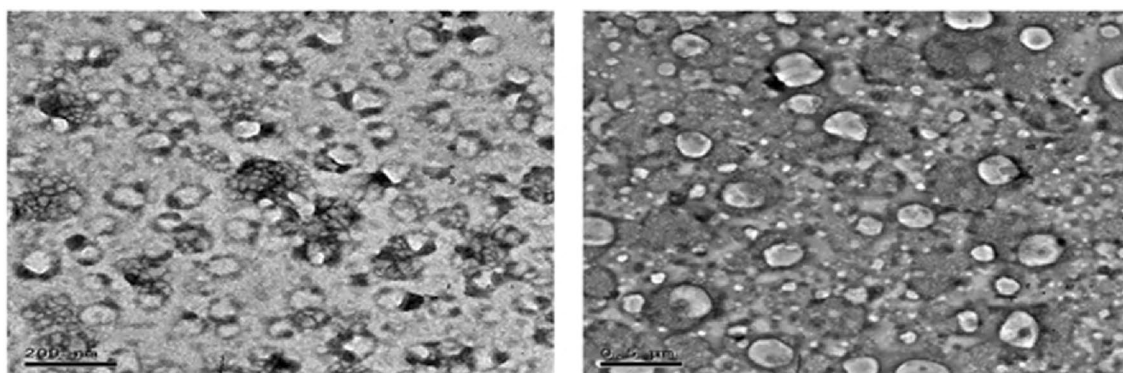


Figure 1. TEM photographs of clove oil nanoemulsion²³.

kg according to the OECD guidelines, and the initial tested dose of CLV-NE in this study was calculated as 5% (100 mg/kg body weight) of the obtained LD50 from acute toxicity test.

Molecular studies

Laddered DNA fragmentation

As shown in Fig. 2 TiO₂-NPs caused dramatic damage to genomic DNA as manifested by the observed smeared and fragmented DNA on agarose gel in comparison to the intact pattern of control genomic DNA. However, oral coadministration of CLV-NE with TiO₂-NPs declined TiO₂-NPs induced DNA damage and restored the integrity of genomic DNA as shown by the re-appearance of an intact genomic DNA pattern of mice given TiO₂-NPs with CLV-NE (Fig. 2 & Fig. S1). Moreover, oral administration of CLV-NE does not cause any changes in the pattern of genomic DNA compared to that of negative control mice.

Expression of p53 gene

As shown in Table 2 oral co-administration of CLV-NE simultaneously with TiO₂-NPs (1.16 ± 0.08^a) caused statistically significant ($P < 0.001$) decrease in the expression level of p53 gene compared to their highly upregulated expression level in mice orally given TiO₂-NPs alone (2.93 ± 0.59^b) and even reached the negative control level. On the other hand, no significant differences were observed in the p53 expression level after administration of CLV-NE contrasted to the negative control (Table 2).

Biochemical studies

In the present study, the activities of superoxide dismutase (SOD) and glutathione peroxidase (Gpx) enzymes and MDA level revealed a non-significant difference between the control and CLV-NE given group in the stomach, kidney, and liver tissues as shown in Figs. 3, 4, 5. Administration of TiO₂-NPs induced significant ($P < 0.001$) reduction in SOD and Gpx activities and significant elevation in the MDA level with respect to the control values. However, simultaneous coadministration of CLV-NE with TiO₂-NPs significantly ($P < 0.001$) ameliorated

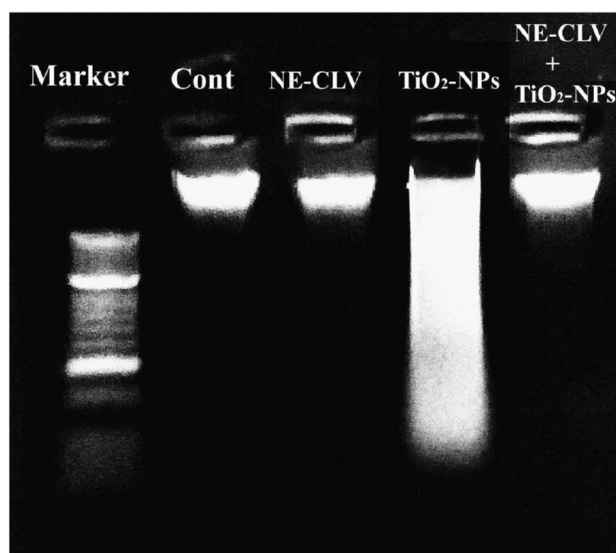


Figure 2. Pattern of the electrophoresed genomic DNA of the negative control group and mice administered clove nano-emulsion (CLV-NE) or/and TiO₂-NPs.

Group	Treatment (dose mg/kg)	P53
Negative control	0	1.00 ± 0.00^a
CLV-NE	100 mg	1.11 ± 0.01^a
TiO ₂ -NPs	50 mg	2.93 ± 0.59^b
CLV-NE + TiO ₂ -NPs	100 mg + 50 mg	1.16 ± 0.08^a
One way analysis of variance (ANOVA)		$F = 28.47$ $P < 0.001$

Table 2. p53 gene expression levels in the stomach tissues in negative control group and groups administered either by clove nano-emulsion or/and TiO₂-NPs. Results are expressed as mean \pm SD. One Way Analysis of Variance was used for analysis followed by Duncan's test to test the similarity between the control and three treated groups. Means with different letters indicated statistically significant difference between the compared groups in the same column.

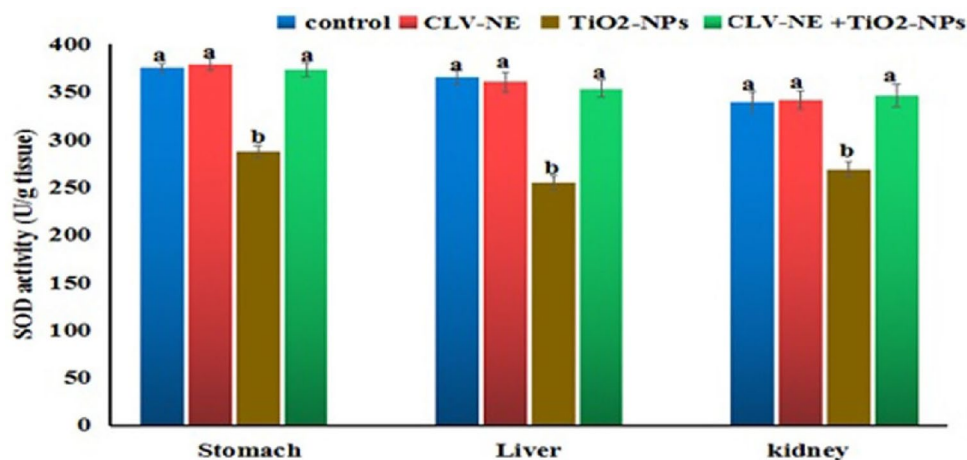


Figure 3. SOD activity in the stomach, liver and kidney tissue homogenate of mice in the control and various treated groups. CLV-NE = clove oil nanoemulsion, TiO₂-NPs = titanium dioxide nanoparticles, TiO₂-NPs + CLV-NE = titanium dioxide nanoparticles plus clove oil nanoemulsion. The data expressed as mean ± SD.

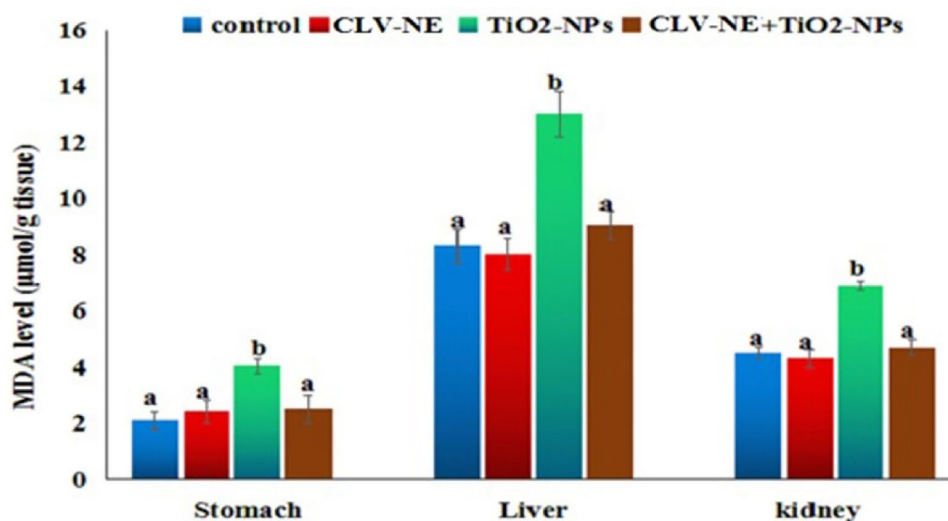


Figure 4. GPX activity in the stomach, liver and kidney tissue homogenate of mice in the control and various treated groups. CLV-NE = clove oil nanoemulsion, TiO₂-NPs = titanium dioxide nanoparticles, TiO₂-NPs + CLV-NE = titanium dioxide nanoparticles plus clove oil nanoemulsion. The data expressed as mean ± SD.

TiO₂-NPs induced disruption in the MDA level and activities of antioxidant SOD and GPx enzymes up to the normal control level (Figs. 3, 4, 5).

Histopathological examination

The histology of stomach tissues demonstrated that control group and CLV-NE groups revealed normal stomach layers with normal mucosa, submucosa, and muscosa. However, treatment with TiO₂-NPs caused diffused mucosal degeneration, and the desquamated mucosal epithelium, together with submucosal blood vessel congestion. While, administration of CLV-NE simultaneously with TiO₂-NPs regenerated the mucosal layer and diminished TiO₂-NPs induced degeneration (Fig. 6). Regarding the liver tissue, normal hepatic parenchyma with normal hepatocytes, central vein, and blood sinusoids were seen in the negative control and CLV-NE groups. Whereas TiO₂-NPs multiple administration resulted in diffusion and degeneration of hepatocytes which were regenerated after coadministration of CLV-NE with TiO₂-NPs (Fig. 7). For kidney tissue, Control group and CLV-NE groups showed normal renal parenchyma, healthy renal glomeruli, and renal tubules. Toxic nephrosis with the diffuse degeneration and necrosis of renal tubules were detected after the administration of TiO₂-NPs. While coadministration of CLV-NE simultaneously with TiO₂-NPs regenerated the renal tubules and showed no degeneration or necrosis (Fig. 8).

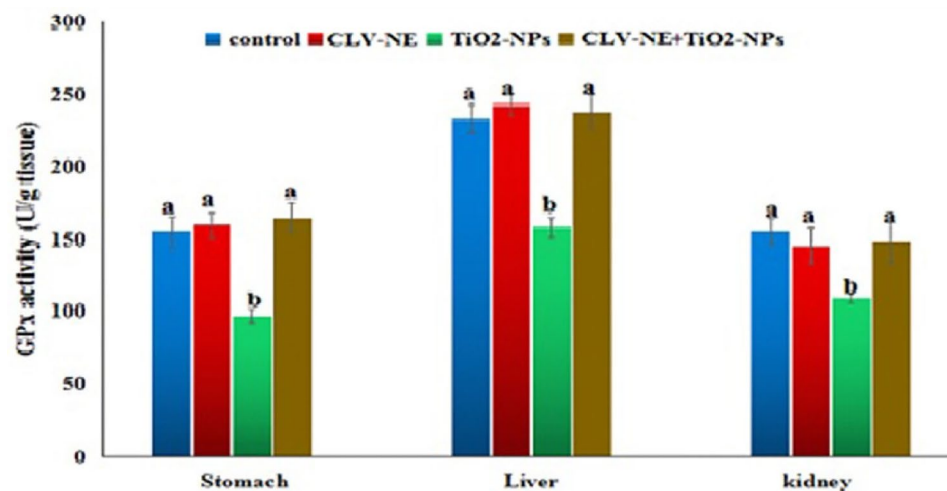


Figure 5. MDA levels in the stomach, liver and kidney tissue homogenate of mice in the control and various treated groups. CLV-NE = clove oil nanoemulsion, TiO₂-NPs = titanium dioxide nanoparticles, TiO₂-NPs + CLV-NE = titanium dioxide nanoparticles plus clove oil nanoemulsion. The data expressed as mean \pm SD.

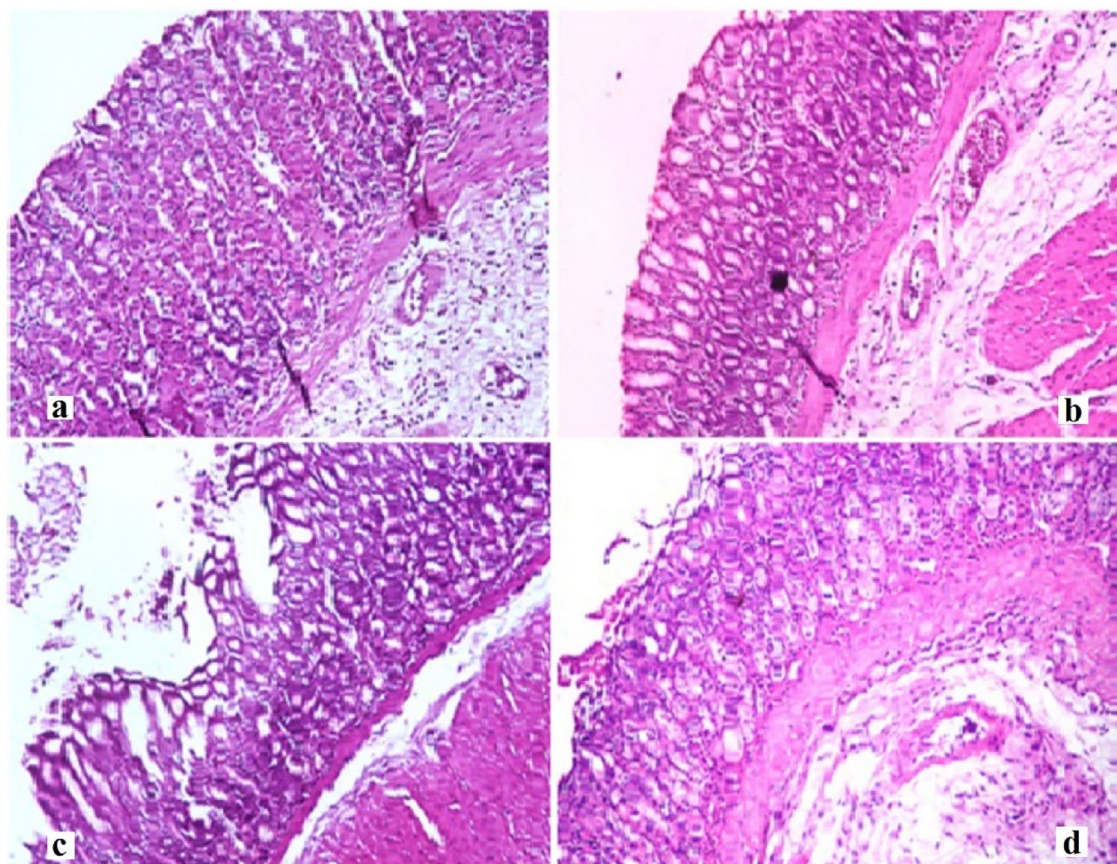


Figure 6. Photomicrographs of Stomach from different experimental groups stained with Hematoxylin & Eosin showing (X200). (a) negative control group, (b) clove oil nanoemulsion group, (c) titanium dioxide nanoparticles and (d) titanium dioxide nanoparticles plus clove oil nanoemulsion.

Discussion

TiO₂-NPs recently used in widespread applications in industry and medicine. However, the safety of TiO₂-NPs exposure to stomach, liver and kidney is still unclear. CLV-NE has been proven for its effectiveness as natural antioxidant product. The present study has investigated the potential toxicity of TiO₂-NPs in term of histological,

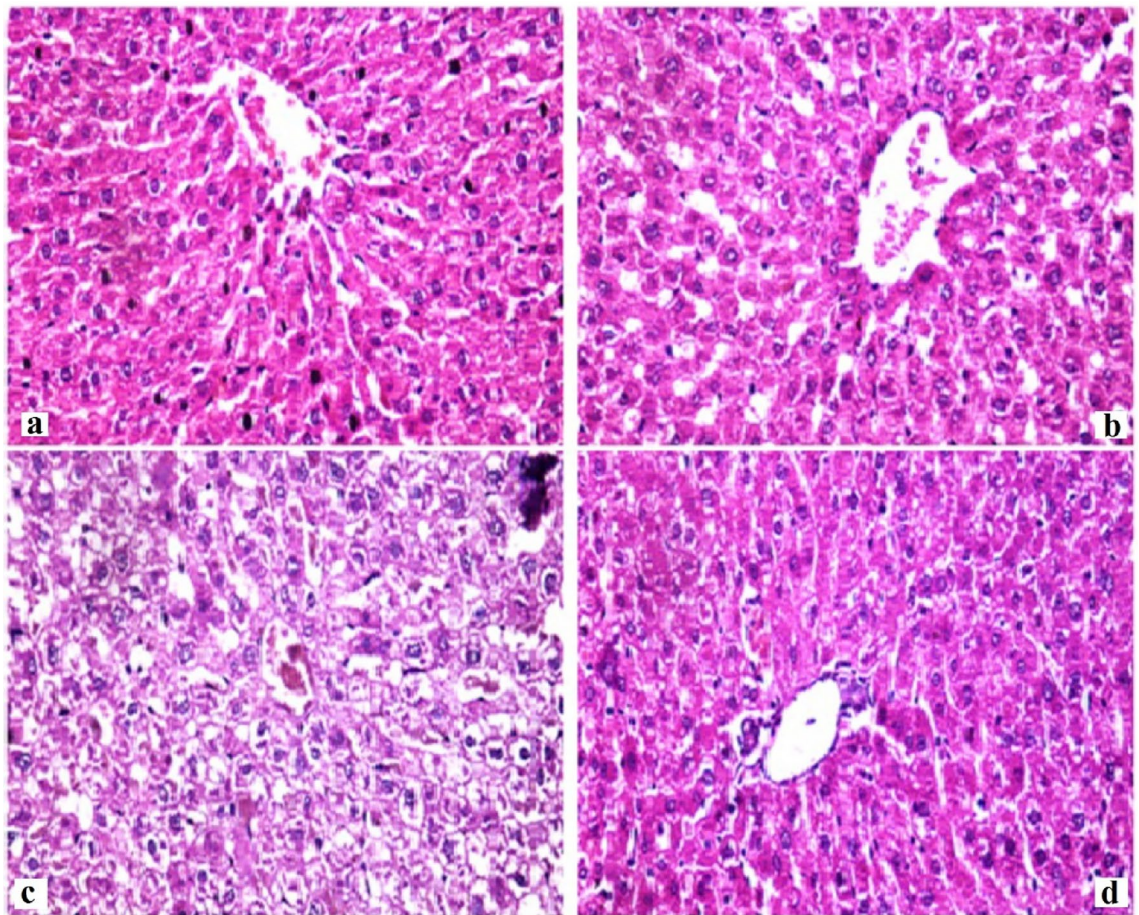


Figure 7. Photomicrographs of Livers from different experimental groups stained with Hematoxylin & Eosin showing (X400). (a) negative control group, (b) clove oil nanoemulsion group, (c) titanium dioxide nanoparticles and (d) titanium dioxide nanoparticles plus clove oil nanoemulsion.

molecular, and biochemical examinations, and the efficiency of using CLV-NE as a protective agent against TiO₂-NPs induced toxicity.

The present data demonstrated that TiO₂-NPs induced DNA damage and oxidative stress in the tested tissues as manifested by the significant elevations in tail length, %DNA and tail moment along with a concomitant marked reduction in the activity of antioxidant enzymes (SOD and GPX) and significant elevation in the MDA level noticed after TiO₂-NPs. These results indicated marked DNA damage induction by TiO₂-NPs chronic administration through a high ROS production and diminished scavenging capacity of the antioxidant enzymes. These findings agree with several authors who confirmed the induction of oxidative DNA damage due to oxidative stress after TiO₂-NPs administration in brain, lung, Hippocampus, and kidney of mice^{23,29–31}, and liver tissue of rat³². These studies also demonstrated that TiO₂-NPs have elevated the induction of reactive oxygen species (ROS), and consequently increased the MDA production, which induced the oxidative DNA damage^{22,33–35}. MDA destroy some functional and structural proteins leading to cell death³⁶.

Moreover, ROS specially the highly cytotoxic free radical, singlet oxygen (¹O₂) mediates cell killing by increasing DNA damage and lipid oxidation due to its interaction with unsaturated fatty acids on the surface of the mitochondrial membrane, inducing necrosis, autophagy and apoptosis^{36,37}. Abundant literature demonstrated that TiO₂-NPs potential genotoxicity may be attributed either to their interference with the structural or functional enzymes of DNA repair system or to the direct interaction with DNA-related proteins or DNA itself^{33,38}. Sun et al.³⁹ stated that TiO₂-NPs affect the proteins of endoplasmic reticulum, enter the nucleus or block its pores and then interact with DNA causing an elevation of the apoptotic related genes, oxidative stress and cytokines genes^{39,40}.

Apoptosis has been detected in our study via the elevation of p53 expression level and significant DNA fragmentation after treatment with TiO₂-NPs. The findings presented here agree with previous studies shown that TiO₂-NPs induced elevation of apoptotic and oxidative stress genes in liver and intestine tissues in rat after 7 days of administration^{41,42}. It has concluded that the large surface area of TiO₂-NPs caused an increased production of reactive oxygen species which resulted in imbalanced homeostasis between oxidation and anti-oxidative activities that implicated with the oxidative stress, genotoxicity and cytotoxicity⁴³.

The increased production of ROS after administration of TiO₂-NPs may be rationalized by activation of heme oxygenase 1 through p38-Nrf-2 signaling pathway or attachment of TiO₂-NPs to prion protein the plasma

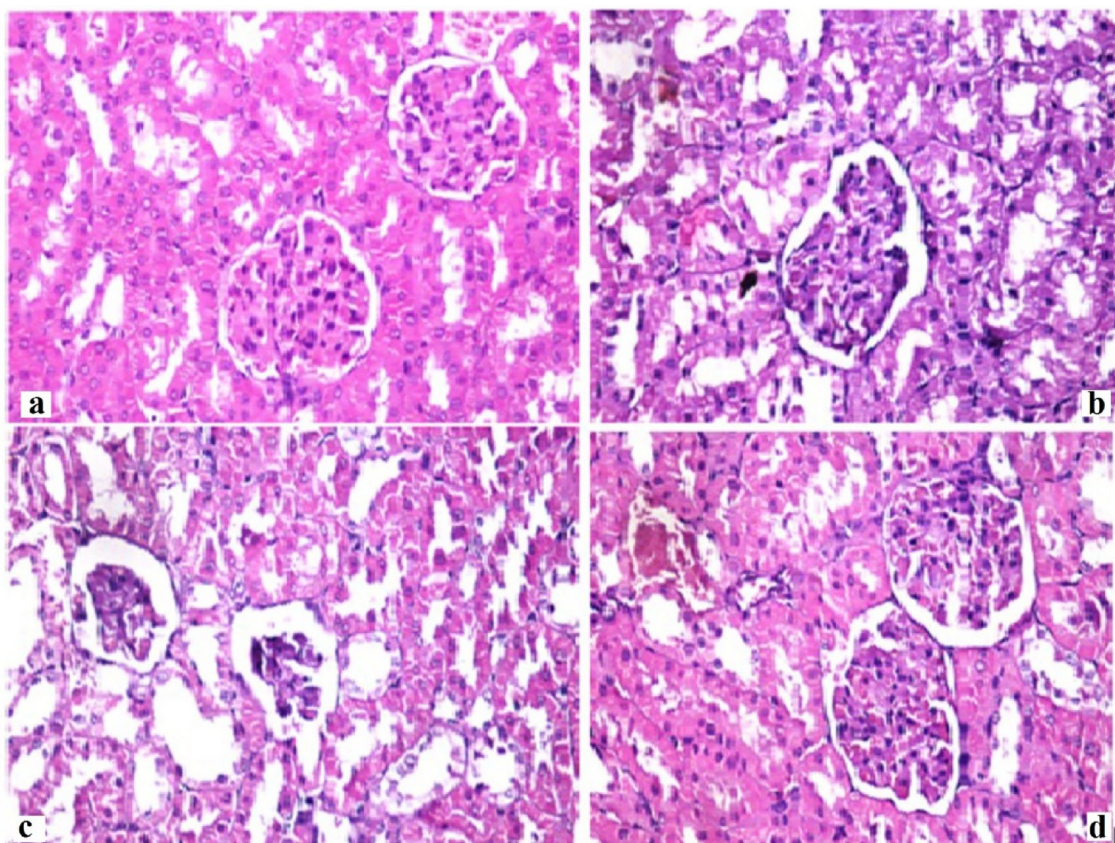


Figure 8. Photomicrographs of Kidneys from different experimental groups stained with Hematoxylin & Eosin (X400). (a) negative control group, (b) clove oil nanoemulsion group, (c) titanium dioxide nanoparticles and (d) titanium dioxide nanoparticles plus clove oil nanoemulsion.

membrane protein which distorts prion signaling function. This prompts the activation of NADPH oxidase via prion-dependent pathway and consequently increases the production of ROS that changes redox equilibrium^{44,45}.

Further, cytoplasmic ROS are known to induce pathophysiological diseases in several tissues, which have indicated in this study by the abnormal architecture noticed in the stomach, liver and kidney tissues of mice orally given TiO₂-NPs alone. These results may be attributed to the high production of ROS, inflammation, and finally cell death due to death of mitochondria⁴⁶. Beside the ordinary way of ROS production, accumulation of mitochondrial ROS due to impairment of its scavenger mechanism leads to organelle dysfunction and cell death³⁶. Taken together, TiO₂-NPs can induce toxicity by three mechanisms; by ROS induction, or lipid peroxidation of the cell membrane and its damaged due to attachment of nanoparticles on the cell surface by electrostatic force, or by TiO₂-NP attachment to intracellular organelles like mitochondria after cell membrane destruction¹⁰.

On the other hand, co-administration of CLV-NE, a powerful antioxidant natural product mitigated TiO₂-NPs induced DNA damage and oxidative stress along with restoring the normal tissues' structure. This ameliorative effect of CLV-NE was demonstrated by the remarkable reductions in the DNA damage level and MDA level along with significant increases in the activity of antioxidant SOD and Gpx enzymes, and may be explained by its high content of anti-inflammatory and antioxidant eugenol derivative compounds that prevent prostaglandin synthesis and chemotaxis of neutrophil^{47,48}.

In addition, eugenol has an effective inhibitory effect on protein denaturation that may stabilize the cell membrane and inhibiting the seepage of intracellular enzymes⁴⁹. More importantly, eugenol reported as an effective antioxidant therapeutic agent that could decrease the ROS in cardiovascular disease by increasing the thioredoxin reductase activity⁵⁰. Decreasing the oxidative stress restore the scavenging activities of SOD and GPX which in turn abolishes the oxidative DNA damage and histopathological injuries induced by TiO₂-NPs. abolishment of TiO₂-NPs induced tissues injuries was detected in this study by the noticed regeneration and restoration of normal tissues' architecture after coadministration of CLV-NE with TiO₂-NPs. In addition, emulsification extended the clove oil' scavenging time of free radicals due to increased surface area⁵¹. According to polar paradox theory, high efficiency of antioxidant activity of clove oil emulsion is due to its non-polarity in O/W emulsion^{51,52}.

Conclusion

In conclusion, administration of TiO₂-NPs induced oxidative DNA damage with increased ROS production that overwhelmed the capacity of SOD and GPX and consequently resulted in elevated level of MDA, all led to apoptosis induction and histopathological changes in stomach, liver, and kidney. However, Concurrent administration of CLV-NE with TiO₂-NPs sustained the genomic DNA integrity and normal tissues architecture through its

free radicals scavenging capacity that overcome MDA production restore the activity of antioxidant enzymes to normal level. Further research is still needed to explore the efficiency of using CLV-NE as a protective or even a therapeutic agent to manage the toxic effects of TiO₂-NPs use for chronic period.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

- Musial, J., Krakowiak, R., Mlynarczyk, D. T., Goslinski, T. & Stanisz, B. J. J. N. Titanium dioxide nanoparticles in food and personal care products—What do we know about their safety?. *Nanomaterials* **10**(6), 1110 (2020).
- Li, D. *et al.* Influence of particle geometry on gastrointestinal transit and absorption following oral administration. *ACS Appl. Mater. Interfaces* **9**(49), 42492–42502 (2017).
- Chen, Z. *et al.* Tissue-specific oxidative stress and element distribution after oral exposure to titanium dioxide nanoparticles in rats. *Nanoscale* **12**(38), 20033–20046 (2020).
- Liu, R. *et al.* Small-sized titanium dioxide nanoparticles mediate immune toxicity in rat pulmonary alveolar macrophages in vivo. *J. Nanosci. Nanotechnol.* **10**(8), 5161–5169 (2010).
- Gui, S. *et al.* Molecular mechanism of kidney injury of mice caused by exposure to titanium dioxide nanoparticles. *J. Hazard. Mater.* **195**, 365–370 (2011).
- Cui, Y. *et al.* Gene expression in liver injury caused by long-term exposure to titanium dioxide nanoparticles in mice. *Toxicol. Sci.* **128**(1), 171–185 (2012).
- Helmy, A. M., Sharaf-El-Din, N. A., Abd-El-Moneim, R. A. & Rostom, D. M. Histological study of the renal cortical proximal and distal tubules in adult male albino rats following prolonged administration of titanium dioxide nanoparticles and the possible protective role of l-carnosine. *Egypt. J. Histol.* **38**(1), 126–142 (2015).
- Abdel-Halim, K. Y., Osman, S. R., Abuzeid, M. A., El-Danasoury, H. T. & Khozimy, A. M. Apoptotic and histopathological defects enhanced by titanium dioxide nanoparticles in male mice after short-term exposure. *Toxicol. Rep.* **9**, 1331–1346 (2022).
- Falck, G. *et al.* Genotoxic effects of nanosized and fine TiO₂. *Hum. Exp. Toxicol.* **28**(6–7), 339–352 (2009).
- Hou, J. *et al.* Toxicity and mechanisms of action of titanium dioxide nanoparticles in living organisms. *J. Environ. Sci.* **75**, 40–53 (2019).
- Najmi, Z. *et al.* Screening of different essential oils based on their physicochemical and microbiological properties to preserve red fruits and improve their shelf life. *Foods* **12**(2), 332 (2023).
- Kumar, S. & Narasimhan, B. J. C. C. J. Therapeutic potential of heterocyclic pyrimidine scaffolds. *Chem. Cent. J.* **12**(1), 1–29 (2018).
- Pontes, N. H. L. *et al.* Impact of eugenol on in vivo model of 6-hydroxydopamine-induced oxidative stress. *Free Radic. Res.* **55**(5), 556–568 (2021).
- Fangjun, L. & Zhijia, Y. Tumor suppressive roles of eugenol in human lung cancer cells. *Thorac. Cancer* **9**(1), 25–29 (2018).
- Zhang, L.-L., Zhang, L.-F., Xu, J.-G. & Hu, Q.-P. Comparison study on antioxidant, DNA damage protective and antibacterial activities of eugenol and isoeugenol against several foodborne pathogens. *Food Nutr. Res.* **61**(1), 1353356 (2017).
- Al-Trad, B., Alkhateeb, H., Alsmadi, W. & Al-Zoubi, M. Eugenol ameliorates insulin resistance, oxidative stress and inflammation in high fat-diet/streptozotocin-induced diabetic rat. *Life Sci.* **216**, 183–188 (2019).
- Fathy, M. *et al.* Eugenol exerts apoptotic effect and modulates the sensitivity of HeLa cells to cisplatin and radiation. *Molecules* **24**(21), 3979 (2019).
- Jafri, H., Khan, M. S. A. & Ahmad, I. In vitro efficacy of eugenol in inhibiting single and mixed-biofilms of drug-resistant strains of *Candida albicans* and *Streptococcus mutans*. *Phytomedicine* **54**, 206–213 (2019).
- Purkait, S., Bhattacharya, A., Bag, A. & Chattopadhyay, R. Evaluation of antibiofilm efficacy of essential oil components β-caryophyllene, cinnamaldehyde and eugenol alone and in combination against biofilm formation and preformed biofilms of *Listeria monocytogenes* and *Salmonella typhimurium*. *Lett. Appl. Microbiol.* **71**(2), 195–202 (2020).
- Costa, L., Moreira, J., de Godoy Menezes, I., Dutra, V. & de Almeida, A. Antibiotic resistance profiles and activity of clove essential oil (*Syzygium aromaticum*) against *Pseudomonas aeruginosa* isolated of canine otitis. *Vet. World* **15**(10), 2499–2505 (2022), Abstract.
- Zhao, R. *et al.* Cutoff Ostwald ripening stability of eugenol-in-water emulsion by co-stabilization method and antibacterial activity evaluation. *Food Hydrocoll.* **107**, 105925 (2020).
- Mohamed, H. R. H. Estimation of TiO₂ nanoparticle-induced genotoxicity persistence and possible chronic gastritis-induction in mice. *Food Chem. Toxicol.* **83**, 76–83 (2015).
- El-Shamy, S.A.E.-M. Efficiency of clove oil nanoemulsion in modulating titanium dioxide-induced some disorders in the lung of the mice. *Egypt. Acad. J. Biol. Sci.* **14**(1), 511–522 (2022).
- Sriram, M. I., Kanth, S. B. M., Kalishwaralal, K. & Gurunathan, S. Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. *Int. J. Nanomed.* **5**, 753–762 (2012).
- Gutierrez, M. I. *et al.* Infrequent p53 mutation in mouse tumors with deregulated myc. *Cancer Res.* **52**, 1032–1035 (1992).
- Nishikimi, M., Roa, N., & Yogi, K. J. B. B. R. C. Measurement of superoxide dismutase. **46**, 849–854 (1972).
- Paglia, D. E. & Valentine, W. N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70**(1), 158–169 (1967).
- Ohkawa, H., Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**(2), 351–358 (1979).
- El-Ghor, A., Noshay, M. M., Galal, A. & Mohamed, H. R. H. Normalization of nano-sized TiO₂-induced clastogenicity, genotoxicity and mutagenicity by chlorophyllin administration in mice brain, liver, and bone marrow cells. *Toxicol. Sci.* **142**(1), 21–32 (2014).
- Habibi, P. *et al.* Thermal stress and TiO₂ nanoparticle-induced oxidative DNA damage and apoptosis in mouse hippocampus. *Environ. Sci. Pollut. Res.* **29**(60), 90128–90139 (2022).
- Nassar, S. A. E. A., Abdeljawad, A. M., Al Gazeery, A. S. A. & Aluin, N. M. Nephrotoxicity induced by titanium dioxide nanoparticles (TiO₂ NPs) in albino mice and the possible protective role of vitamin E (A histological and molecular study). *Bull. Fac. Sci. Zagazig Univ.* **2022**(4), 70–78 (2023).
- Sallam, M. F. *et al.* Improvement of the antioxidant activity of thyme essential oil against biosynthesized titanium dioxide nanoparticles-induced oxidative stress, DNA damage, and disturbances in gene expression in vivo. *J. Trace Elem. Med. Biol.* **73**, 127024 (2022).
- Singh, N. *et al.* NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials* **30**(23–24), 3891–3914 (2009).

34. Shukla, R. K. *et al.* ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicol. Vitro* **25**(1), 231–241 (2011).
35. Faddah, L., Abdel, B. & Al-Rasheed, N. Biochemical responses of nanosize Titanium dioxide in the heart of rats following administration of idepenone and quercetin. *Afr. J. Pharm. Pharmacol.* **7**(38), 2639–2651 (2013).
36. Forrester, S. J., Kikuchi, D. S., Hernandez, M. S., Xu, Q. & Griendling, K. K. Reactive oxygen species in metabolic and inflammatory signaling. *Circ. Res.* **122**(6), 877–902 (2018).
37. Wan, M. T. & Lin, J. Y. Current evidence and applications of photodynamic therapy in dermatology. *Clin. Cosmet. Investig. Dermatol.* **7**, 145 (2014).
38. Karlsson, H. L. The comet assay in nanotoxicology research. *Anal. Bioanal. Chem.* **398**(2), 651–666 (2010).
39. Sun, Q. *et al.* Pulmotoxicological effects caused by long-term titanium dioxide nanoparticles exposure in mice. *J. Hazard. Mater.* **235**, 47–53 (2012).
40. Meena, R., Pal, R., Narayan Pradhan, S. & Paulraj, R. Comparative study of TiO₂ and TiSiO₄ nanoparticles induced oxidative stress and apoptosis of HEK-293 cells. *Adv. Mater. Lett.* **3**(6), 459–465 (2012).
41. Abbasi-Oshaghi, E., Mirzaei, F. & Pourjafar, M. NLRP3 inflammasome, oxidative stress, and apoptosis induced in the intestine and liver of rats treated with titanium dioxide nanoparticles: in vivo and in vitro study. *Int. J. Nanomed.* **14**, 1919 (2019).
42. Hagag, S., Ghareeb, S., Ragheb, D., Ashour, M. B. & Elsheikh, A. (2022) Gene expression analysis in male rat exposure to titanium dioxide nanoparticles.
43. Ghareeb, S., Ragheb, D., El-Sheakh, A. & Ashour, M.-B.A. Potential toxic effects of exposure to titanium silicon oxide nanoparticles in male rats. *Int. J. Environ. Res. Public Health* **19**(4), 2029 (2022).
44. Ze, Y. *et al.* Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice. *Chemosphere* **92**(9), 1183–1189 (2013).
45. Ribeiro, L. W. *et al.* Titanium dioxide and carbon black nanoparticles disrupt neuronal homeostasis via excessive activation of cellular prion protein signaling. *Part. Fibre Toxicol.* **19**(1), 1–22 (2022).
46. Woo, J.-H. *et al.* Polypropylene nanoplastic exposure leads to lung inflammation through p38-mediated NF-κB pathway due to mitochondrial damage. *Part. Fibre Toxicol.* **20**(1), 1–17 (2023).
47. El-Saber Batiha, G. *et al.* *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules* **10**(2), 202 (2020).
48. Selvakumar, P. Pharmacological, bioactive chemical constituents and medicinal uses of *Syzygium Aromaticum* L.
49. Dinurrosifa, R. S. & Indriyanti, E. J. J. A green synthesis of acetyl eugenol by sonochemical method and potential as anti-inflammatory in-vitro. *7*(3), 324–332.
50. Guenzet, A. *et al.* Reduction of plasma homocysteine and 8-iso-prostaglandin F2a concentrations in hypercholesterolemic rats treated with clove essential oil. *South Asian J. Exp. Biol.* **12**(6), 782–788 (2022).
51. Jacobsen, C., Let, M. B., Nielsen, N. S. & Meyer, A. S. Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: A comparative evaluation. *Trends Food Sci. Technol.* **19**(2), 76–93 (2008).
52. Mohamed, H. R. H. Attenuation of nano-TiO₂ induced genotoxicity, mutagenicity and apoptosis by chlorophyllin in mice cardiac cells. *Int. J. Sci. Res.* **3**(6), 2625–2636 (2014).

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Author contributions

H.R.H. Mohamed: conceptualization, methodology; data collection and analysis; writing—original draft preparation, H.M.; writing, review and editing. All authors have read and agreed to the published version of the manuscript.

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Competing interests

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Additional information

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