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RESEARCH ARTICLE

Impact of titanium dioxide nanoparticles on lead acetate-induced genotoxicity in the major histocompatibility complex region and 16S rRNA sequence in mice

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Abstract

This manuscript aimed to study the effect of co-exposure of both lead acetate and titanium dioxide nanoparticles (**(**TiO_2 NPs) on the major histocompatibility complex (*Mhc*) region and 16S rRNA sequence in vivo. Twenty-four male albino Swiss mice have randomly separated into four groups; Group 1 (control): was received only distilled water. Groups 2-4 received lead acetate (400 mg/kg body weight, orally by gavage for 15 consecutive days) + either distilled water (orally), or $ZnCl_2$ (4 mg/kg body weight, intraperitoneal injection), or titanium deoxidize nanoparticles TiO_2 (200 mg/kg body weight, intraperitoneal injection) for additional 15 consecutive days, respectively. According to the results of 16S rRNA sequence that the highest P-distance value (0.002) found between control group and both (lead acetate and lead acetate+TiO_2)-treated groups which reflected the genetic effects of both lead acetate and TiO_2 NPs. The results of microsatellites revealed that the exposure to lead acetate affected the genetic structure, where the genetic similarity between control group and lead acetate-treated group was 0.83, while zinc chloride decrease lead acetate-induced genotoxicity where the genetic similarity was 0.88 comparing to the control group. Moreover, the co-exposure to lead acetate and TiO_2 NPs led to more genotoxicity and DNA damage, that is clear in the genetic similarity between control group and lead acetate+TiO_2-treated group which was 0.74.

Keywords: 16S rRNA; Genotoxicity; Lead acetate; *Mhc* genes; Titanium dioxide (TiO_2) NPs.

1. Introduction

Due to the recent technological development, a lot of our daily life applications uses heavy metals, which stress the environment by accumulating different hazardous substances and contribute to increase in the probability of environmental pollution (Basketter *et al.*, 1999). Lead is classified as a possible human carcinogenic by the international agency for research cancer (IARC) and one of the most dangerous poisons and pollutants found in our environment (Sharma and Street, 1980; Mello *et al.*, 1998). Several industries released lead and its compounds which are regarded as a pollutant of worldwide concern. This makes the in vivo testing of its mutagenic potential critical (Fahmy, 1999). Concerning this point, many studies on humans and animals have reached similar results, where behavioral effects that were caused by lead are seen in rats at approximately the same blood levels that responsible for sever deficits in humans (Annau, 1990; Davis *et al.*, 1990; Needleman *et al.*, 1990). It was found that exposure to lead in the environment increases the toxic effects in the various organ of the body. Also, it was found in laboratory animals that were treated with lead there was accumulates of lead in the kidney (Aziz *et al.*, 2012).

Titanium dioxide TiO_2 is a distinguished semiconductor with very interesting properties, which enabled it to be one of the leader NPs in all daily life applications. TiO_2 NPs have a band gaps 3.2 eV for anatase, 3.02 eV for a rutile and 2.96 eV for a brookite phases. Because of its high stability,

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low cost and non-toxicity , TiO_2 is used as a photovoltaic cell in dye sensitized solar cells (Pichot *et al.*, 2000; Saito *et al.*, 2004), hydrogen production in photocatalytic splitting of water, hydrogen production from natural seawater, separate evolution of H_2 and O_2 from water under visible light irradiation (Matsuoka *et al.*, 2007), hydrogen storage in reversible storage of H_2 on nanotubular TiO_2 arrays (Bavykin *et al.*, 2004; Pillai *et al.*, 2006). In the biomedical field it used in Cancer prevention and treatment, Photodynamic therapy, drug delivery system, cell imaging, biosensing , genetic engineering and medical implants (Amij, 2007; Mansoori *et al.*, 2007).

The prevalently use of nanomaterials is associated with releasing wastes including nano particles, which could pollute the environment and may cause toxic effects and health problems to human and animals. Titanium dioxide (TiO_2) have been widely used as a new kind of photocatalyst, antiultraviolet light agents and photoelectric effect agents, in commercial business such as anti-aging research, white pigments preparation, cosmetic applications and water purification techniques (Park *et al.*, 2006).

The widely used TiO_2 NPs in the different industrial products cause a state controversy about the effect of NPs on the environment and human health (Weir *et al.*, 2012). NPs can enter the body and freely cross biological barriers due to their smaller size and unique properties. Many studies revealed that inhaled/injected nanoparticles enter the circulator system and transfer to various organs and tissues (Takenaka *et al.*, 2001; Kreyling *et al.*, 2002), in turn, they could accumulate and damage them (Mohamed and Hussien, 2016).

TiO_2 NPs is found in three crystalline forms, anatase, rutile, and brookite (Wu *et al.*, 2010; Markowska-Szczupak *et al.*, 2011). The brookite is little used, while the anatase and rutile forms have natural and industrial concern. Generally, anatase is more harmful than rutile and, regrettably, being used amply (Markowska-Szczupak *et al.*, 2011; Iswarya *et al.*, 2015). For these reasons it is important to study the interaction of TiO_2 NPs and lead acetate in vivo and assess their degree of damage.

For several years, zinc has been known to be having the efficiency to retard oxidative processes (Powell, 2000). Zinc has essential roles in cell metabolism, it is binding a broad range of proteins as well as it is need for the activity of several key regulatory proteins (Leoni *et al*., 2014).

The major histocompatibility complex (*MHC*) is a multigene family encoding cell-surface glycoprotein that mediates both humoral and cell-mediated immune responses. The *Mhc* region of the mouse exists on chromosome 17 and comprising 2000–4000kb of DNA. This region contains genes classified as class I, class II, and class III. Some of the class I as well as the class II genes are the most polymorphic genes known (Hood *et al.*, 1983; Huang and Yu, 2003). *MHC* genes are very polymorphic because of the diversity and balancing natural selection, but the accurate nature of this selection is vague (Apanius *et al.*, 1997).

Genes in the Vertebrate major histocompatibility complex (*MHC*) have a significant role in immune recognition and the starting of immune responses (Klein, 1986). Because of the important role of *MHC* in immunity, it is commonly presupposed that the selective pressures affecting *MHC* variation come directly from diseases infectious (Hughes and Nei, 1992; Slade and McCallum, 1992; Potts and Slev, 1995).

Microsatellites are referred to as short tandem repeats of simple sequences with repeat lengths of six base-pairs or less (Beckman and Weber, 1992; Hearne *et al.*, 1992). They take place frequently and are numerous and quite spread throughout the whole genome (Hearne *et al.*, 1992; O'Reilly and Wright, 1995). Due to diversity in the number of repeat units, these arrays display great polymorphism, so the interest of microsatellites was considerably increased. Generally, the long of microsatellites are less than 100 bp, and their polymorphisms can be efficiently analyzed by polymerase chain reaction followed by agarose gel electrophoresis (Weber and May, 1989). Therefore, microsatellites are perfect markers on chromosomes that used as anchors for genetic analyses (Love *et al.*, 1990; Dietrich *et al.*, 1992). It can be reported that all microsatellite markers are

highly delicate, need less time and economical sound technique as well as they are handling simply (Purohit *et al.*, 2015).

The 16S gene is considered as the ideal molecular clock and it is prevalently use in phylogenetic, evolutionary, and taxonomic studies. The great number of suitable primers and the presence of large volumes of partial sequences of the 16S gene in the many databases led to clear classification (Sokefun, 2017).

Our efforts in this work were related to study the genotoxicity of lead acetate on the microsatellite loci of *Mhc* region and 16S rRNA sequence in mice as well as study the impact of TiO_2 NPs in these variations. Also, to compare these influences with the effects of zinc chloride against the genotoxicity in *Mch* region.

2. Materials and Methods

2.1. Preparation of TiO_2 NPs

TiO_2 NPs were prepared hydrothermally at temperature 130 ºC. The preparation procedure followed four steps. Briefly, 2g of commercial TiO_2 was mixed with 80 mL of 10M NaOH under stirring for 10 min. then the mixture was placed into a Teflon lined autoclave and heated at 130 ºC for 24 h. The autoclave was allowed to cool naturally to room temperature. The obtained product was collected and washed with diluted HCl (pH 1.6) for 24 h. The acid washed product was washed several times with distilled water until the pH arrived 7. The final product was annealed at 400 ºC for 2 h.

2.2. Characterization of TiO_2 NPs

X-ray diffraction (XRD) measurements were performed using X'Pert PRO-PAN analytical diffractometer with Cu-Kα radiation (λ= 1.54056 A˚) at 40 kV and 30mA. The prepared sample was examined by infrared spectral analysis by using KBr disk technique to evaluate the functional groups of the studied samples. The used spectrometer was (FTIR Model 6100, Jasco-Japan) with a resolution of 4.00 cm-1 and covers the wave number range of 4000 – 400 cm-1. The external features and morphology of the sample was detected by high resolution transmission electron microscope (JEOL, JEM 2100, Japan). UV-visible spectrophotometer (SPECORD 200 PLUS, Analytik Jena, Germany) was used to obtain optical absorption spectra of the TiO_2 sample.

2.3. Chemicals

Lead acetate 3-hydrate, purity 98%, and zinc chloride, purity 98%, were obtained from Gene Tech, Cairo, Egypt.

2.4. Animals

All procedures were approved and done according to the guidelines approved by the Ethics Committee of the Faculty of Science, South Valley University, Qena, Egypt. Twenty-four male albino Swiss mice (about 6-8 weeks old and weighing 21±1gm) were obtained from the College of Veterinary, South Valley University, Qena, Egypt. The animals were kept in cages in animal's laboratory, Faculty of Science, South Valley University, Qena, Egypt, for two weeks under normal condition, and same food and water were provided to all.

2.5. Experiment design

Twenty-four male albino Swiss mice have randomly divided into four groups: with six animals in each group. Group 1 (control): was received only distilled water. Groups 2-4 received lead acetate (400 mg/kg body weight, orally by gavage for 15 sequential days) + either distilled water (orally), or $ZnCl_2$ (4 mg/kg body weight, intraperitoneal injection), or titanium deoxidize nanoparticles TiO_2 (200 mg/kg body weight, intraperitoneal injection) for additional 15 sequential days, respectively. The mice were killed 24 h after the last dose. The kidney tissues were removed and stored in -20ºC until DNA extraction.

2.6. DNA Extraction

By following the manufacturer's guidelines of QIAamp DNA Mini kit (Qiagen, Hidden, Germany), we extracted the DNA from the preserved tissues.

2.7. Large subunit ribosomal RNA gene (16SrRNA) Sequence

2.7.1. PCR Amplification and Sequencing

Polymerase chain reaction (PCR) amplification of genomic DNA was performed using primers according to (Simon *et al*., 1991). PCR achieved for the amplification of isolated DNA using a total of 50 µL containing;1 µL genomic DNA as a template, 1 µL of each forward and reverse primer, 22 µL of nuclease free water and 25 µL of 2X master mix. The PCR amplification programme comprised an initial denaturation at 95 °C for 3 min. followed by 35 cycles; denaturation, annealing and extension for 1 min. at 94 °C, 48 °C and 72°C respectively. Cycling was terminated with a 10-min extension at 72 °C. The PCR products were separated and visualized by electrophoresis on a 1.5% ethidium bromide-stained agarose gel. The PCR amplification yielded a single band in each group. The sequences were submitted to the National Center for Biotechnology Information (GenBank/NCBI) to get the accession numbers.

2.7.2. Phylogenetic analysis

Sequence alignment was implemented using ClustalW (Thompson *et al*., 1994). Phylogenetic analyses were performed with MEGA version 7.0 18 (Kumar *et al*., 2016), using, Neighbour Joining (NJ) method and using 1000 bootstrap iterations (Felsenstein, 1985). Sequence divergences were calculated using Kimura 2-parameter distances (Kimura, 1980).

2.8. Microsatellite loci

Seven microsatellite loci (D17Mit21, D17Mit28, D17Mit33, D17Mit83, D17Mit103, D17Mit214, and D17Nds3) very related to the *Mhc* genes were utilized. These seven microsatellites were found on chromosome 17 between 18.00 and 19.50 centimorgan from the centromere and bounded by the K and L genes where most of the highly polymorphic antigen-presenting *Mhc* loci have been instituted (Meagher and Potts, 1997).

PCR achieved for the amplification of isolated DNA using a total of 25µ; comprising, 0.5 µL of each forward and reverse primer, 1 µL genomic DNA, 10.5 µL of nuclease free water and 12.5 µL of master mix. The PCR amplification programme comprised an initial denaturation at 95 °C for 3 min. followed by 30 cycles; denaturation, annealing and extension for 1 min. at 94 °C, at primer specific annealing temperature (50ºC-57ºC) and 72°C respectively. Cycling was terminated with a 7-min extension at 72 °C. The PCR products were separated and visualized by 1.5 % ethidium bromide-stained agarose gel.

2.8.1. Statistical analysis

Numbers of observed alleles and expected heterozygosity were achieved by POPGEN 1.32 software. Average heterozygosity (H.av), Discriminating power (D) and Marker Index (MI) were evaluated by Online Marker Efficiency Calculator iMEC (Amiryousefi *et al*., 2018). The genetic similarity and dendrogram were calculated by PAST software. Polymorphic information contents (PIC) were calculated according to (Bińkowski, 2018). The data was analyzed by the mixed model ANOVA and general linear model using program of SPSS version 16. The Chi-square test was performed to compare conception rates among groups.

3. Results

3.1. X-ray diffraction (XRD)

Structural properties of TiO_2 pattern (Fig. 1) revealed the overall crystalline structure and phase purity of the TiO_2 NPs. All the relatively peaks could be indexed as anatase TiO_2, which are basically in agreement with the reported card (JCPDS No. 21-1272). Although the diffraction peak of brookite B (121) in can also be found, it is much lower than those of anatase phase. No characteristic peaks of other impurities, such as NaCl and Na2TiO3, were observed, which indicates that the product has high purity as confirmed by EDX and EDR *(*Asiah *et al*., 2015; Attar and Hassani, 2015).

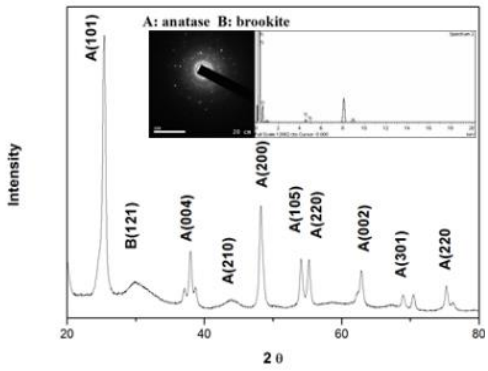

Fig.1. Structural properties of TiO_2 NPs

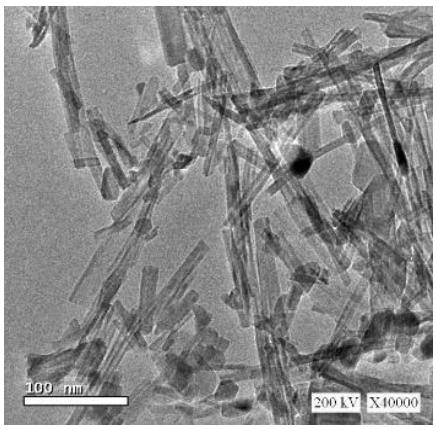

Fig.3. HR-TEM image of TiO_2 NPs

3.2. Fourier transformation Infrared spectroscopy (FT-IR)

Fig. 2 represent FTIR spectrum of TiO_2 NPs three bands were observed, the first broad band appeared between 3800 to 3000 cm-1 belongs to stretching hydroxyl (O-H), representing the water as moisture. The second peak was observed between 1626 and 1638 cm-1, which belongs to the stretching of titanium carboxilate. The third peak between 800 and 450 cm-1 was assigned to the Ti-O stretching bands (Ba-Abbad *et al*., 2012).

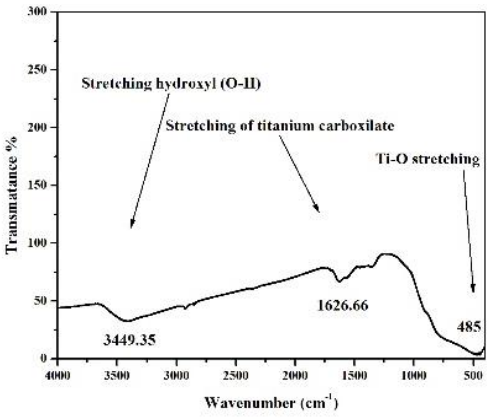

Fig.2. FTIR spectrum of TiO_2 NPs

3.3. High resolution transmission electron microscope (HR-TEM)

The morphology of TiO_2 NPs was imaged using HR-TEM as shown in (Fig. 3). The shape of TiO_2 was a tube-like shape which corresponding to the synthesis conditions (hydrothermal method) (Ba-Abbad *et al*., 2012; Asiah *et al*., 2015).

3.4. Sequence variation using (16S rRNA)

In this study, partial sequences for the mitochondrial 16S rRNA gene in the four groups were determined. There was small size variation among groups, the fragment size ranged from 548 bp to 550 bp. The partial nucleotide sequences of 16S rRNA were deposited in the GenBank under accession numbers (MW054194-MW054197). The final concatenated alignment obtained consisted of 551 bp. Out of them 545 and two were conserved sites and variable sites, respectively. The average A+T content was 61.8, which was higher than the C+G content. The aligned sequences length, A+T contents, C+G contents for each group and their average, were illustrated in (Table 1).

The P-distances among the groups ranged from 0.00 to 0.02 %. Overall, the distance value among all groups was 0.002%. The highest P-distance (0.002) found between control group and both lead acetate-treated group and lead acetate+TiO_2-treated groups which reflected the genetic effects of both lead acetate and TiO_2 NPs. While the lowest value (0.000) found between control group and lead acetate+$ZnCl_2$-treated group (Table 2).

We assessed the degree of support by bootstrap values for each of the clades recovered by Neighbour Joining (NJ) clades. The phylogenetic analysis among the four groups was achieved through Neighbour Joining (NJ) method and revealed that lead acetate+TiO_2-treated group formed a separate cluster, while the remaining groups found in one clad within it, control group and lead acetate+ $ZnCl_2$-treated group formed a sister clade (Figs. 4,5).

Table1. Accession numbers, nucleotide frequencies and their averages of (16S rRNA) gene in the four groups

Group	Accession number	Base pair Length	Nucleotide Number %				A+T Content (%)	C+G Content (%)
			A%	T%	C %	G%		
Control	MW054194.1	548.0	32.7	29.0	18.8	19.5	61.7	38.3
Lead acetate	MW054195.1	548.0	32.7	29.0	19.0	19.3	61.7	38.3
Lead acetate+$ZnCl_2$	MW054196.1	549.0	32.8	29.0	18.7	19.5	61.8	38.2
Lead acetate+TiO_2	MW054197.1	550.0	32.9	29.1	18.5	19.5	62	38
Average %	-	548.8	32.8	29.0	18.7	19.5	61.8	38.2

Table 2. Pairwise distances based on (16S rRNA) gene among the four groups

Group				
MW054194.1 Control		0.002	0.000	0.002
MW054195.1 Lead acetate	0.004		0.002	0.002
MW054196.1 Lead acetate+$ZnCl_2$	0.000	0.004		0.002
MW054197.1 Lead acetate+TiO_2	0.002	0.002	0.002	

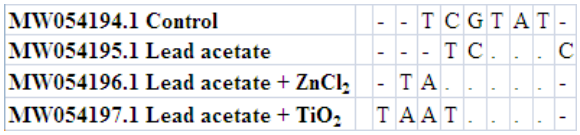

Fig.4. Alignment of partial sequences of (16SrRNA) gene in the four groups. Dots indicate the identical nucleotides and A, T, C, and G indicates the difference nucleotides.

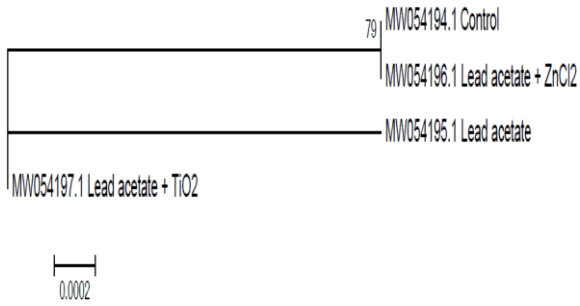

Fig.5. Phylogenetic tree using the Neighbour Joining method among the four groups based on partial sequences of (16S rRNA) gene.

3.5. Microsatellite

For all groups, the numbers of alleles ranged from 7 to 19. The maximum number of alleles was obtained in D17Mit28 locus and minimum number of alleles was found in D17Mit103 locus. Mean of observed number of alleles for all the loci was10.3. The maximum expected heterozygosity was obtained in D17Mit214 and D17Mit103 as 0.49 and the minimum expected heterozygosity was in D17Mit28 as 0.30 with mean of 0.41. The maximum observed heterozygosity was reported in D17Mit83 and D17Mit33 as 0.5 and minimum in D17Mit214 as 0.37 with mean of 0.46. Average heterozygosity was measured maximum in the marker D17Mit214 as 0.0233 and minimum 0.0056 in D17Mit28 with mean of 0.176. The PIC values ranged from 0.24 in D17MitD to 049 in D17Mit214. The mean of PIC value was 0.37. The Marker Index (MI) values among the 7 microsatellite loci ranged from 0.014 (D17Mit28) to 0.046 (D17Mit103), and the average value of MI was 0.034. The Discriminating power (D) values among the 7 microsatellite loci ranged from 0.34 (D17Mit28) to 0.71 (D17Mit103) and the average value of MI was 0.54 (Fig. 6).

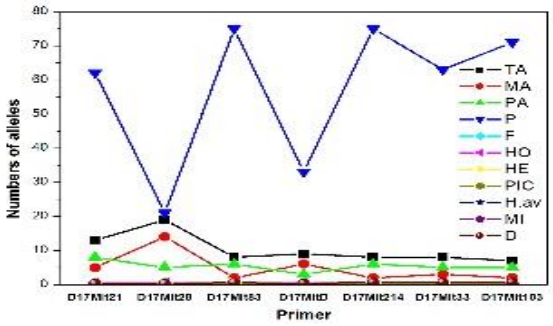

Fig. 6. Primers, total of alleles (TA), monomorphic alleles (MA). Polymorphic allele (PA), % polymorphic (%P), Frequency (F), Heterozygocity index (H_o), Polymorphic Information Content (PIC), Arithmatic mean of H (Hav), Marker Index (MI), Discriminating power. (D).

Table 3. The Similarity matrix UPGMA Jaccard's Coefficient among the four groups

Group	Control	Lead acetate	Lead acetate + $ZnCl_2$	Lead acetate + TiO_2
Control	1.00			
Lead acetate	0.83	1.00		
Lead acetate +$ZnCl_2$	0.88	0.85	1.00	
Lead acetate +TiO_2	0.74	0.75	0.78	1.00

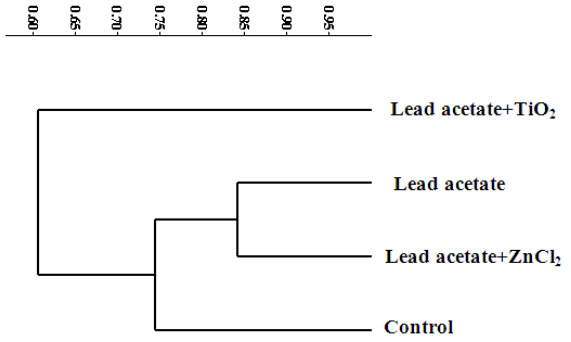

Fig.7. Dendrogram demonstrating the relationship among the four groups based on data recorded from microsatellite.

The genetic similarity of the four groups was calculated by past software. The high genetic similarity value 0.88 was found between control group and lead acetate+$ZnCl_2$-treated group. The low genetic similarity 0.74 was found between control group and lead acetate+TiO_2-treated group (Table 3). The dendrogram consisted of two main clusters. The first main cluster encloses lead acetate+TiO_2-treated group. The second main cluster includes the remaining groups (Fig.7).

4. Discussion

Several studies have been concerned with the genotoxicity effects of TiO_2NPs exposure, including DNA damage, in vitro mammalian chromosomal aberrations and micronuclei development (Landsiedel *et al.*, 2009; Mohamed and Hussien, 2016). Genotoxicity is the ability of a chemical substance to change the genetic material of the cell, and is one of the primary influences of most carcinogens (Xie *et al.*, 2011).

In the mouse, the *Mhc* genomic region existed on chromosome 17, and the genes of this region are commonly classified based on their structure and function into three different classes (I to III) (Kumánovics and Lindahl, 2004). Interestingly, the effect of *MHC* genes is not only limited to immune conditions, but also involved tendency to infectious diseases, malignancies and neuropathologies (Trowsdale, 2011; Howell, 2014). It may be argued that the *MHC* region has been known as the region of the genome that have the greatest number of human disease associations (Trowsdale and Knight, 2013).

In the present investigation, we used seven microsatellites located on chromosome 17 between the K and D loci due to the higher number of alleles in this region. In *M. domestics* strains, the average number of alleles is higher for loci between the K and D loci, than those loci either near or away from this region (Meagher and Potts, 1997).

Of those seven microsatellites, D17Mit21, and D17Mit28 display higher rates of polymorphism than the others. D17Mit21, existed within the intron 3 of the A-beta-2 gene, and D17Mit28, found in the promoter region of the K gene. This result was consistent with the fact that the Aβ gene in the class II family and the K gene in the class I family are among the most polymorphic *Mhc* genes (Hood *et al*., 1983). On the contrary, D17Mit33, revealed less polymorphism degree where it found within the class III C4/Slp hybrid 3 gene. This result was in agreement with the previous data of DNA sequence and allozyme which exhibited low genetic variation at the class III genes, regardless of their being closely linked to genes of class I and II (Hood *et al*., 1983; Klitz *et al*., 1986; Satta *et al*., 1998).

The results of 16S rRNA sequences and microsatellite revealed that lead acetate caused genotoxicity comparing to the control group. Where the lead acetate-treated group was genetically distance from the control group because of the effect of lead acetate. Several studies confirmed these findings; Fahmy (1999) deduced that exposure the mice with lead acetate at the doses 50,100,200 and 400 mg kg-1 b.wt.

increased the percentage of chromosomal aberration significantly in bone-marrow as well as spermatocytes and the higher doses revealed possibility of genotoxicity.

Evidences revealed that lead compounds can contributed in a fention reaction to generate damaging oxygen radicals and can cause DNA strand breaks (Roy and Rossman, 1992). The mechanisms for these genotoxicity may include direct damage to DNA which influence on the stability of chromatin or interacting with repair processes (Johansson and Pellicciari, 1988; Hartwig *et al.*, 1990). In the same context, lead ions could decrease the fidelity of DNA synthesis, as well as cause failure the mechanisms of DNA repairing (Acharya *et al.*, 2003; Ahmed *et al.*, 2012).Kasuba *et al.* (2004) suggested that low doses of lead acetate, which not affect growth and development of rats, cause an identified genome damage in sucking rats exposed to lead both subchronically (for 9 days) or cutely (in single intraperitoneal injection). Exposure to lead acetate crashes balances between prooxidant and antioxidant system which lead to depletion of the cellular antioxidants, and consequently, DNA or protein damage mitochondrial impairment and apoptosis (Hsu and Guo, 2002; Sabath and Robles-Osorio, 2012).

The results of 16S rRNA sequences showed the lowest distance value (0.000) was found between control group and lead acetate+$ZnCl_2$-treated group. The same result was obtained by microsatellite, where the high genetic similarity value 0.88 was found between control group and lead acetate+$ZnCl_2$-treated group. This refers to improvement effect of $ZnCl_2$ which decrease the effects of lead acetate. Badkoobeh *et al.* (2013) and Grüngreiff (2016) declared that zinc participates in several important biological processes and protects the DNA strand from damage.

The co-pollutants of complex mixtures, can affect the biotransformation efficiency at the catalytic, transcriptional and protein levels (Elbekai and El-Kadi, 2005; Regoli *et al.*, 2005; Sorrentino *et al.*, 2005; Gravato *et al.*, 2006; Benedetti *et al.*, 2007). Therefore, the investigations of interactions among toxicants are of essential importance and practical interest in toxicological sciences (Wilson *et al.*, 2007).

In the present work, the treatment of the sample exposure to lead acetate with TiO_2 doses led to more genotoxicity and DNA damage. The genetic similarity between control group and lead acetate+TiO2-treated group was 0.74, which was less than the genetic similarity between control group and lead acetate-treated group 0.83. This reflected the effect of TiO_2 on the genetic structure. Because synergistic or inhibitory effects, cascade, and indirect mechanisms can both reinforce or curb the expected responses from specific pollutants. Therefore, the biological consequences of chemical mixtures require delicate evaluation (Regoli *et al.*, 2005).

5. Conclusion

Our results indicated the sensitivity of using microsatellite loci linked to *Mhc* genes and 16S rRNA sequence in the detection of genotoxicity, which caused by lead acetate and TiO_2 NPs. TiO_2 NPs can absorb metal ions such as lead, and their interaction in vivo give rise to more genetic variations and genotoxicity. The exposure to TiO_2 NPs effect on the genetic structure, that is clear in the genetic similarity between control group and lead acetate+TiO_2-treated group, which was less than the genetic similarity between control group and lead acetate-treated group. Finally, humans exposed daily to TiO_2 compounds by using sunscreens, toothpaste, sweats medications etc. and lead through transportation and industrial sources which may be responsible for a serious genetic problem that should be controlled and taken into account.

6. Conflict of interest

The authors have no conflict of interest to declare.

7. Funding

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8. Statement of animal rights:

All institutional and national guidelines for the care and use of animals were according to the Egyptian Medical Research Ethics Committee (no. 14e126).

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