Kafkas Univ Vet Fak Derg
24 (6): 799-805, 2018
DOI: 10.9775/kvfd.2018.19904

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://submit.vetdergikafkas.org

Research Article

Evaluation of Genotoxic Effects of C_{60} Fullerene-γ-Fe_2O_3 and Multi-Wall Carbon Nanotubes-γ-Fe_2O_3 Nanoparticles

Özlem DEMİRCİ [1,a] Nesrin HAŞİMİ [2,b] Ersin KILINÇ [3,c] Veysel TOLAN [4,d]

[1] Dicle University, Science Faculty, Department of Biology, TR-21280 Diyarbakır - TURKEY
[2] Batman University, School of Health, Department of Nutrition and Dietetics, TR-72060 Batman - TURKEY
[3] Dicle University, Vocational School of Technical Sciences, Department of Chemistry and Chemical Processing Technologies, TR-21280 Diyarbakır - TURKEY
[4] Dicle University, Science Faculty, Department of Molecular Biology and Genetics, TR-21280 Diyarbakır - TURKEY
[a] ORCID: 0000-0001-9511-2010; [b] ORCID: 0000-0003-1367-5624; [c] ORCID: 0000-0001-5223-9919; [d] 0000-0003-0172-6957

Article Code: KVFD-2018-19904 Received: 10.04.2018 Accepted: 19.09.2018 Published Online: 24.09.2018

How to Cite This Article

Demirci Ö, Haşimi N, Kilinç E, Tolan V: Evaluation of genotoxic effects of C_{60} fullerene-γ-Fe_2O_3 and multi-wall carbon nanotubes-γ-Fe_2O_3 nanoparticles. *Kafkas Univ Vet Fak Derg,* 24 (6): 799-805, 2018. DOI: 10.9775/kvfd.2018.19904

Abstract

The use of magnetic nanoparticles in nanomedicine applications has increased significantly in recent years. Genotoxic evaluation of the nanomaterials used for this purpose is therefore very important. In our study, the genotoxic effect of C_{60} fullerene-γ-Fe_2O_3 and multi-wall carbon nanotubes-γ-Fe_2O_3 magnetic nanoparticles over a wide concentration range (0.1, 1.0, 5.0, 10.0, 25.0, 50.0, and 100.0 µg/plate) was investigated using the Bacterial Reverse Mutation Test. These magnetic nanoparticles did not cause genetic damage to *Salmonella typhimurium* TA100 and TA98 in the presence and absence of metabolic activation. Due to the rapid increase in the presence of nanoparticles in our daily lives, mutagenicity and toxicity data related to nanoparticles are quite valuable. For this reason, *in vivo* and *in vitro* studies that allow for effective evaluation of these compounds is of the utmost importance.

Keywords: *Genotoxicity, Nanotoxicity, Multi-wall carbon nanotubes-γ-Fe_2O_3, C_{60} Fullerene-γ-Fe_2O_3, Salmonella/Microsome mutagenicity assay*

C_{60} Fullerene-γ-Fe_2O_3 ve Çok Duvarlı Karbon Nanotüpler-γ-Fe_2O_3 Nanopartiküllerinin Genotoksik Etkilerinin Değerlendirilmesi

Öz

Nanotıp uygulamalarında manyetik nano parçacıkların kullanılması son yıllarda önemli ölçüde artmıştır. Bu amaçla kullanılan nano malzemelerin genotoksik değerlendirilmesi bu nedenle çok önemlidir. Çalışmamızda, C_{60} fullerene-γ-Fe_2O_3 ve çok duvarlı karbon nanotüpler γ-Fe_2O_3 manyetik nanopartiküllerin geniş bir konsantrasyon aralığında (0.1, 1.0, 5.0, 10.0, 25.0, 50.0 ve 100.0 µg/plaka) genotoksik etkisi Bakteriyel Geri Mutasyon Testi kullanılarak araştırıldı. Bu manyetik nanopartiküller, metabolik aktivasyonun varlığında ve yokluğunda *Salmonella typhimurium* TA100 ve TA98'de genetik hasara neden olmamıştır. Günlük yaşamlarımızda nanopartiküllerin varlığındaki hızlı artış nedeniyle, nanopartiküller ile ilgili mutajenite ve toksisite verileri oldukça değerlidir. Bu nedenle, bu bileşiklerin etkili bir şekilde değerlendirilmesine olanak sağlayan *in vivo* ve *in vitro* çalışmalar oldukça önemlidir.

Anahtar sözcükler: *Genotoksisite, Nanotoksisite, Çok duvarlı karbon nanotüpler-γ-Fe_2O_3, C_{60} fullerene-γ-Fe_2O_3, Salmonella/Mikrozom mutajenite testi*

INTRODUCTION

Nanotechnology deals with the production of nanometer-scale (1-100nm) materials with the appropriate size and features to suit the potential area of use [1,2]. Nanotechnology products such as polymeric nanomaterials, fullerenes, single-walled carbon nanotubes, multi-walled carbon nanotubes, magnetic nanoparticles, and quantum dots have a wide range of applications [3]. As the particle size of a material decreases to the nanometer range, its physical and chemical properties vary considerably from those of its larger counterparts [4]. With these developments, it

İletişim (Correspondence)
+90 412 2411000/3197
ozdem22@gmail.com

is important to examine the effects of nanomaterials on living organisms, such as their toxicity and mutagenicity.

It is difficult to design multifunctional nanoparticles with the necessary properties to be effective for both the diagnosis and treatment of diseases. One of the important precursors in this area is magnetic nanoparticles. We have studied the mutagenesis of γ-Fe_2O_3 magnetic nanoparticles containing multi-wall carbon nanotubes and C_{60} fullerene, which are potentially useful in nanomedicine. In the recent past, nanomedical applications of magnetic nanoparticles have been investigated due to their superparamagnetic moments with high magnetic saturation [5].

Superparamagnetic iron oxide nanoparticles (SPION) with different surface chemistries are used in many *in vivo* nanomedical applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery, and in-cell separation [6].

Carbon nanotubes (CNTs) are nanomaterials with very specific physicochemical properties that can be used as biosensors in nanotechnology and medical applications, as new vehicles for diagnosis and treatment of diseases, and as molecular carriers for drug delivery [7]. However, research on CNTs is steadily on living organisms as new applications for these materials as medical devices for treatment and diagnosis are discovered [8]. However, little work has been done on the effects of CNTs on human and other living things, and this effect varies depending on the nature of the CNTs, the duration of exposure, and the dose [9].

Fullerenes, especially C_{60}, have very good physical and electrochemical properties for use in medical fields. Fullerenes may have different activity areas, including antiviral activity as an inhibitor of HIV protease and antioxidant activity as a radical scavenger. However, when fullerenes are exposed to light, singlet oxygen is produced, which can lead to DNA damage by direct electron transfer. At the same time, fullerenes can be used as a tool in gene and drug delivery. Although the nanoparticle properties such as the suitability for injection and high accumulation potential for target organs are important for medical applications, the most important property is nontoxicity [10]. Genotoxicity and cytotoxicity studies are common due to increased concern about the toxic potential of nanoparticles [11].

In our study, the genotoxic effect of C_{60}-γ-Fe_2O_3 and MWCNT-γ-Fe_2O_3 nanoparticles at a wide range of concentrations was tested using the OECD test guidelines 471 (Bacterial Reverse Mutation Test). The principle of this test is to detect mutations that inhibit the synthesis of certain essential amino acids and cause recurrence of mutations previously formed in test strains [12]. In the Bacterial Reverse Mutation Test *(*Salmonella/microsome mutagenicity assay), the S9 mix is used for metabolic activation. The S9 mixture acts as an imitator of the oxidative system responsible for the oxidative, peroxidative, and reductive metabolisms of various biological or chemical substances containing cytochrome P-450. Metabolic activation (S9) is therefore a valuable method for detecting changes in mutagenicity as a result of biotransformation of a compound during *in vitro* studies [13-15].

The main objective of this work is to determine the genotoxicity of C_{60} fullerene-γ-Fe_2O_3 (C_{60}-γ-Fe_2O_3) and multi-wall carbon nanotubes-γ-Fe_2O_3 (MWCNT-γ-Fe_2O_3) nanoparticles, which will be used in biomedical applications such as drug transport.

MATERIAL and METHODS

Nanoparticles

The C_{60}-γ-Fe_2O_3 and MWCNT-γ-Fe_2O_3 nanoparticles used in this work were synthesized by Dr. Kilinc [3,16]. In this study, Sodium azide was used as a positive control and DMSO/water was used as a negative control to determine the mutagenicity of the nanoparticles. The positive controls used in this study were 1 µg/plate Sodium azide (NaN_3) for TA100, 1.5 µg/plate 2-amino-fluorene (2-AF) for TA98.

Instrumentation

Concentration of Fe was measured by ICP-OES (Perkin Elmer, Optima 2100 DV) at a wavelength of 238.204 nm. Infrared spectra of cMWCNT and MNP at 4000-400 cm intervals were recorded by FT-IR (Mattson Model 1000). Model P525 Vibrating Sample Magnetometer (VSM) was used as a physical property measurement system (PPMS). To obtain SEM and HR-TEM images of MNPs, a LEO-Evo 40XVP scanning electron microscope and a Jeol JEM 2100 FHR TEM were used at 200 kV with a probe size below 0.5nm [3,16].

Synthesis of cMWCNT-γ-Fe_2O_3 Magnetic Nanoparticle

1.25g MWCNT was refluxed in 50 mL 1.0 mol/L HNO_3 for 24 h in order to expose the raw MWCNT to strong acidic conditions for carboxylation. Then, the dispersion was exposed to a mixture of H_2SO_4 and HNO_3 (3:1, v/v) at 30°C for 5.0 h. The cMWCNT obtained by filtration was then dried at 85°C for 24 h.

1.25g of MWCNT was refluxed in 50 mL of 1.0 mol/L HNO_3 for a day. Then it was dispersed in a mixture of H_2SO_4 and HNO_3 (3:1, v/v) and sonicated at 30°C for 5.0 h. Then, cMWCNT (oxidized and shortened) was filtered and dried in oven at 85°C for a day. $FeCl_3{\cdot}6H_2O$ and $FeCl_2$ (mole ratio 2:1) were dissolved in 40 mL of distilled water and 0.1g of cMWCNT was added to it by vigorously stirring. 30 mL of NH_3 was added dropwise for a time of 60 min at 80°C. Resulting black solution was filtered and cMWCNT-γ-Fe_2O_3 as black precipitate was washed with distilled water until it was neutral. Then, it was dried in oven at 90°C for a day [16].

Synthesis of C60-γ-Fe_2O_3 Magnetic Nanoparticle

γ-Fe_2O_3 (0.36g) was added to the solution of 0.17g of C60 fullerene dissolved in 100.0 mL of toluene. The mixture was sonicated for 5.0 min at 30°C and vigorously stirred for 3 days at room temperature. $FeCl_3.6H_2O$ and $FeCl_2$, at the molar ratio of 2:1, were dissolved in distilled water and stirred in a 100.0 mL three necked flask. 30.0 mL of 5% NH_4OH solution was added dropwise at 75°C with vigorous stirring for about 2.0 h under nitrogen purge. It was subsequently washed with distilled water, toluene, and absolute ethanol until the C60 absorption peak disappeared by monitoring UV-VIS spectra at 554nm [3].

Characterization of cMWCNT-γ-Fe_2O_3 and C60-γ-Fe_2O_3 Magnetic Nanoparticle

Approximately 0.01g of cMWCNT and cMWCNT-γ-Fe_2O_3 were weighed. First 3.0 mL concentrated HCl and then 0.5 mL H_2O_2 and 1.0 mL HNO_3 added into the beaker and heated to dryness. The resulting cMWCNT-γ-Fe_2O_3 MNPs and cMWCNT were dissolved in 50 and 5.0 mL of 1.0 mol/L HNO_3, respectively. The Fe concentrations in the cMWCNT-γ-Fe_2O_3 magnetic nanoparticle and raw cMWCNT from were measured by ICP-OES. Surface functionality were investigated by FT-IR spectra. The chemical structure of γ-Fe_2O_3 nanoparticles functionalized with cMWCNT was investigated by XRD. The magnetic saturation value of MNPs were determined using VSM. Macro-structures of nanomaterials were determined by SEM while morphology and microstructures were determined using HR-TEM [3,16].

Bacteria Strains

The strains TA98 and TA100 of *Salmonella typhimurium* were purchased from the Salmonella genetic stock center at University of Calgary, Canada and genetically controlled [17]. Salmonella strains were used with or without the S9 mix [18].

S9-based Metabolic Activation System

S9 was prepared using a mixture of metabolic activator, phosphate buffer (0.2 M), 130 µL deionized water, S9 fraction 100 µL, KCl (0.33 M), $MgCl_2$ (0.1 M), NADP (0.1M), and glucose-6-phosphate (0.1M). The supernatant from liver homogenate of rats exposed to phenobarbital was used for elution of S9 fractions [18,19].

Bacterial Reverse Mutation Assay

Bacterial reverse mutation assays were performed according to Maron and Ames [18]. Basically, histidine-independent and histidine-dependent mutations were detected using *S. typhimurium* TA98 and TA100 test strains in the presence or absence of S9 mix.

S. typhimurium TA98 and TA100 test strains supplied as lyophilized strains were diluted by adding 1 mL of nutrient broth under aseptic conditions. Then, the diluted culture was transferred into 4 mL of nutrient broth. Single colony planting on nutrient-scored plaques by taking a drop of diluted cultures. Plates were maintained at +4°C for genotyping strains. Control tests performed for mutant strains before mutation tests were performed include control of histidine requirement, control of R factor, control of Rfa mutation, control of *uvr*B mutation and control of the number of spontaneous return colonies. Master plates, which can be stored at +4°C, were also prepared simultaneously with the control of genetic properties. Plates containing selective nutrient media prepared for the control of each property were plated on the same column with sterile toothpicks from the test strips, which were reduced by one colony, to the plate of MGA, MGA + histidine/biotin, ampicillinous nutrient agar and two nutrient agar (for UV and master plaque) respectively. The plates were incubated at 37°C. After incubation, the colonies bearing the full genetic traits were labeled on the master plate and stored at +4°C for use in culture preparation. Positive mutagen control used to detect bacterial responses to known standard mutagenes was made in parallel with the essential experiment. *S. typhimurium* TA98 was used in the presence of the 2-Nitrofluorene S9 mixture, an indirect mutant for the strain, and in the absence of the direct mutant 2-Nitrofluorene S9 mixture. Sodium azide was used as a direct mutagen for *S. typhimurium* TA100 in the presence and absence of S9 mixture. The 'standard plate incorporation' method was used when running the Ames *Salmonella*/microsomal test system. In this technique; Two different experiments were carried out in the presence and absence of S9. In the mutagenicity/antimutagenicity studies performed with *S. typhimurium* standard test strains, it is predicted that 1-2x10^9 Colony forming unit (CFU) will be one mL of bacterial cultures. For the experiment, first night culture was prepared. The master platelet colony was inoculated into 40 mL of liquid medium and left in the shaker (1-2x10^9 cfu/mL, OD540=0.1-0.2) at 37°C at 120 rpm for 11-13 h. In the experiments in the absence of S9, 100 mL of soft agar, previously liquidified in a hot water bath and adjusted to a temperature of 45-50°C., were distributed in 2 mL of sterile glass tubes of 2 mL each with the addition of 10 mL of 0.5mM histidine/biotin solution. Then 0.1 mL of bacterial culture and 0.1 mL test compound (different concentrations of nanoparticules) were added to the tubes and vortexed for 3 seconds at low speed before spreading to plates containing MGA at room temperature. The mixing-pouring-spreading process is carried out so that the soft agar does not freeze and spread over the entire surface of the plaque, not exceeding 20 sec. The same procedure was applied to positive and negative controls. In experiments in the presence of S9, 100 mL of a soft agar, previously liquidified in a hot water bath and adjusted to a temperature of 45-50°C, was added to 10 mL of a 0.5mM histidine/biotin solution and 2 mL of sterile 13×100mm glass tubes It was distributed.

Antimutagenicity experiments were performed in the absence of S9 with *S. typhimurium* TA98 and TA100 strains.

In the antimutagenicity experiments, the overnight culture was firstly prepared as described in the mutagenicity test. 100 mL of a soft agar, which was liquidated in a hot water bath and adjusted to a temperature of 45-50°C, was distributed in sterile glass tubes of 2×3 mL of sterile glass. Then 0.1 mL of bacterial culture, 0.1 mL of mutagen (dounomycine for TA98, sodium azide for TA100), 0.1mL of test compounds at different concentrations and 500 µL of phosphate buffer were added to 2 mL of the top agar containing 0.5 mM histidine/biotin were added to the tubes and vortexed for 3 seconds at low speed before spreading to plates containing MGA (minimal glucose plates). Plates were allowed to incubate for 48-72 h at 37°C. The number of colonies formed after the incubation was recorded. Each dose was tested in parallel on three plates and three independent experiments were performed at different times. The decrease in the number of colonies was calculated according to the following formula as percent inhibition.

For the antimutagenicity assays, the inhibition % was calculated according the formula given below.

(Inhibition % = [1–T/M] ×100)

Where T is the number of revertants per plate in the presence of mutagen, and the test sample, and M is the number of revertants per plate in the positive control. The antimutagenic effect (% inhibition) between 25-40% defined as moderate antimutagenicity, 40% or more as strong antimutagenicity, and 25% or less inhibition as no antimutagenicity [20].

Statistical Analysis

All experiments were carried out in triplicate and the results are expressed as means ± standard deviation (SD). Statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's multiple comparison post-test to verify the significance of a positive response. SPSS software version 14.0 (Illinois, USA) was used for statistical tests, and a p value of $P<0.05$ was considered statistically significant.

RESULTS

Results of the Salmonella microsome test are given in *Table 1* and *Table 2*. Mutagenic assays of both MWCNT-γ-Fe_2O_3 and C_{60}-γ-Fe_2O_3 using a wide range of concentrations (100, 50, 25, 10, 5, 1, and 0.1 µg/plate). MWCNT-γ-Fe_2O_3 did not increase the number of revertant colonies in S. *typhimurium* TA98 and TA100 with and without the metabolic activation mix (S9) *(Table 1)*. Similarly, there was no significant change in the number of revertant colonies in *S. typhimurium* TA98 and TA100 used to investigate the mutagenic potential of C_{60}-γ-Fe_2O_3, both in absence and in presence of the metabolic activation system (S9) *(Table 2)*. However, Sodium azide (NaN_3; for TA100) and 2-amino-fluorene (2-AF; for TA98) used as positive controls significantly increased the number of revertant colonies statistically. Genotoxicity testing is considered a valuable tool for evaluating the carcinogenic risk of nanoparticles [12]. However, a single test cannot identify all relevant genotoxic substances. Therefore, several *in vitro* and *in vivo* tests are typically used to assess genotoxicity. Spontaneous mutational DNA damage in TA98 strain is reverted to wild-type by specific mechanisms of frameshift (templated mutations at CG sequence), which do not occur in TA100 (base substitution at CG sequence).

C_{60} fullerene-γ-Fe_2O_3 [3] and MWCNT-γ-Fe_2O_3 [16] were successfully used adsorptions of flurbiprofen, a non-steroidal anti-inflammatory drug and harmane, one of the most potent tremor producing β-carboline alkaloids. By considering, the results it could be concluded that both of nanomaterials could be used in biomedical application include adsorption and controlled release of drug as nanocarriers.

Table 1. *Spontaneous revertant colonies induced by MWCNT-γ-Fe_2O_3 to S. typhimurium (TA 98, TA 100) with and without metabolic activation (S9)*

Dose (µg/plate)	TA100		TA98	
	-S9	+S9	-S9	+S9
0	89.6±3.4	91.0±12.0	35.3±4.5	33.0±2.0
0.1	100.6±13.0	98.0±12.3	27.3±1.5	36.6±5.5
1	81.3±7.5	98.6±5.1	32.3±5.7	38.3±8.7
5	88.0±0	96.3±10.4	30.3±2.5	40.0±11.2
10	87.3±9.5	102.6±7.5	33.6±9.7	31.3±9.0
25	72.3±5.5	93.6±5.6	38.0±8.0	37.3±4.6
50	87.0±6.0	86.0±13.0	34.0±2.0	30.3±4.1
100	85.0±2.0	88.0±3.4	38.3±2.0	36.0±4.6
DMSO/water	89.0±6.8	89.6±8.0	35.3±4.2	44.3±4.8
Positive Control	960.6±30.4[b]	1615±45.9[b]	368.6±37.8[c]	704.3±24.5[c]

All values are expressed as mean±standard deviation (SD). *[a] DMSO/water, [b] Sodium azide (1 g/plate) (Positive control), [c] 2-Nitrofluorene (2 g/plate) (Positive control), * P<0.05 (ANOVA+Dunnett's multiple comparison post test)*

Table 2. *Spontaneous revertant colonies induced by C60-γ-Fe_2O_3 to S. typhimurium (TA 98, TA 100) with and without metabolic activation (S9)*

Dose (µg/plate)	TA100		TA98	
	-S9	+S9	-S9	+S9
0	89.6±6.0	91.6±8.3	30.3±4.5	44.6±2.0
0.1	67.0±5.5	92.3±11.2	38.6±11.7	39.3±3.7
1	81.3±8.5	70.3±6.8	37.6±10.6	35.0±5.5
5	85.0±9.8	89.3±8.6	34.6±8.5	33.6±6.1
10	117.3±29.8	80.0±11.0	36.3±2.0	32.0±2.0
25	119.0±13.4	92.3±10.6	31.6±3.7	47.0±11.5
50	87.6±4.9	88.0±5.2	34.6±4.6	35.6±5.5
100	88.6±16.0	91.0±4.0	38.3±3.2	34.3±3.5
DMSO/water	89.0±6.8	89.6±8.0	35.3±4.2	44.3±4.8
Positive control	960.6±30.4[b]	1615±45.9[b]	368.6±37.8[c]	704.3±24.5[c]

*All values are expressed as mean±standard deviation (SD). [a] DMSO/water, [b] Sodium azide (1 g/plate)(Positive control), [c] 2-Nitrofluorene (2 g/plate) (Positive control), * P<0.05 (ANOVA + Dunnett's multiple comparison post test)*

DISCUSSION

Recent biomedical studies have shown that magnetic nanoparticles may be an important tool for *in vivo* and *in vitro* applications. Most magnetic nanoparticles contain superparamagnetic iron oxides such as magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). For medical use, it is very important that these particles do not show any toxic effects [21]. In this study, C_{60}-γ-Fe_2O_3 and MWCNT-γ-Fe_2O_3 nanoparticles were investigated for their genotoxic potential. The nanoparticles of superparamagnetic iron oxide nanoparticles used in nanomedicine should have diameters of 5-100 nm to ensure similarity to biological macromolecules and for cell compatibility.

In our study, we used nanoparticles appropriate for medical applications, although the two have different chemical structures. Therefore, this study on genotoxic potential of test samples is very important. Carbon nanotubes can exhibit toxic effects at different levels due to their different sizes and properties, such as different surfaces [22]. However, it may be suggested that similarities between the high aspect ratio nanoparticles (HARNs) and asbestos result in similar toxic potentials, as suggested by the HARNs theory [23]. According to the previous studies as the size of the nanoparticle decreases, the penetration and thus possibility of the toxicity increases [24,25]. Nanoparticle penetration mechanisms are thought to occur by means of adhesive interaction or passive diffusion without a specific receptor on their surface. Electrostatic charges, steric interactions, Van der Waals interactions or interfacial tension effects; can provide penetration of the nanoparticles without vesicles [26,27]. Penetration of the nanoparticles into the cell can occur without the utilization of phagosomes [28]. Previous studies have indicated that C_{60} molecules penetrate through the nuclear membrane after penetration into the cell. However, it has been demonstrated that fullerenes inhibit the activity of HIV protease by binding to the active site, which is a basic enzyme for HIV virus [29]. Nanoparticles used in our study were observed by TEM to have spherical sizes less than 10 nm and 5 nm for C_{60}-γ-Fe_2O_3 and MWCNT-γ-Fe_2O_3, respectively [3,16]. Although the nanoparticle sizes in our study were quite small, their physical and chemical should be evaluated in terms of toxicity.

The detailed mechanisms of nanoparticle-induced genotoxicity are not completely understood and it is furthermore not clear if there are any nano-specific effects on DNA [30]. The "nano-specific effect" means a mechanism of toxic action especially in particles whose initial sizes change 1-100 nm while not associated with those of different sizes and similar chemical compound. Genotoxicity mediated by particle can be categorized in either "primary genotoxicity" or "secondary genotoxicity". The former intends to self-genotoxicity from the nanoparticles while the latter intends to increase in genotoxicity induced by reactive oxygen species (ROS) accumulated in course of particle-revealed inflammation [31]. Not only *in vivo* but also *in vitro* studies have indicated that ROS are produced by nanoparticles of diverse compounds (fullerenes, carbon nanotubes, quantum dots, and automobile exhaust). They cause oxidative damage in cellular components such as lipid peroxidation, protein carbonylations, DNA oxidation, interference in signaling functions, and modulations in gene transcription [32,33].

Previous studies of genotoxicity using the bacterial reverse mutation assay have shown that the increase in the number of returning colonies in bacteria exposed to multi-wall carbon nanotubes is not significant and does not cause mutagenic effects [1,19,22]. Similarly, in this study, the MWCNT-γ-Fe_2O_3 magnetic nanoparticles did not cause mutation under any conditions tested genotoxicity of *(Table 1)*. Jia et al.[34] compared the genotoxic potential of single-wall nanotubes (SWNTs), multi-wall nanotubes

(with diameters ranging from 10 to 20 nm), and C_{60} fullerenes, and determined that C_{60} fullerene and multi-wall nanotubes showed lower cytotoxicity than single-wall nanotubes based on MTT assays. Based on these results, it can be concluded that the cobalt and iron traces of the MWCNT-γ-Fe_2O_3 magnetic nanoparticles we use do not generate genotoxic responses in this test system.

Fullerenes, especially C_{60} attractants, have widespread use in nanomedicine due to their physical and chemical properties [35-37]. Studies investigating whether cytotoxic C_{60} fullerene (C_{60}) is useful for various mammalian cells are available in the literature [38-40]. Studies on the genotoxic effect of C_{60} with the bacterial reverse mutation test (AMES) are also available in the literature [1,37,41]. Shinohara et al.[41] investigated the genotoxic effect of C_{60} nanoparticles by the Bacterial Reverse Mutation Test and found that the number of revertant colonies in the group exposed to C_{60} was less than two times that of the negative control, even with metabolic activation. The negative control of the number of return colonies caused by mutagenic acceptance of a test sample should be more than two-fold [37].

However, studies on the evaluation of the mutagenesis of magnetic γ-Fe_2O_3 (metal oxide) nanoparticles using AMES bioassay are limited [4,42]. Whereas the magnetic forms of nanomaterials are used for many nanomedical applications, Pan et al.[4] evaluated the mutagenesis of different metal oxide nanoparticles (Al_2O_3, Co_3O_4, CuO, TiO_2, and ZnO) by the Bacterial Reverse Mutation Assay. Their results showed that these metal oxide nanoparticles do not exhibit mutagenicity in the absence of S9 metabolic activation. However, in the presence of S9 activation, CuO, TiO_2, and ZnO showed mutagenic potential at different levels for some bacterial strains. In our study, it was observed that there was no significant change in the number of colonies returning by metabolic activation (S9 mix) of C_{60}-γ-Fe_2O_3 nanoparticles *(Table 2)*. This suggests that the nanoparticles used in the study do not cause oxidative or peroxidative effects. These results are reliable, given the authors' experience in the field [3,16]. C_{60} and MWCNT will continue to be valuable additions in the field of medicine, especially since Fe_2O_3-containing nanoparticles are magnetic and do not cause mutagenicity.

As a results of this study, the C_{60}-γ-Fe_2O_3 and MWCNT-γ-Fe_2O_3 magnetic nanoparticles tested in *Salmonella* microsome mutagenicity test have not been shown any muatagenic effect. Although these negative results observed in *Salmonella* microsome mutagenicity test, the further investigation needs to be done. The genotoxicity of C_{60}-γ-Fe_2O_3 and MWCNT-γ-Fe_2O_3 magnetic nanoparticles obtained both provide an overview of the current study and provide useful information for future investigations.

REFERENCES

1. Kim JS, Lee K, Lee YH, Cho HS, Kim KH, Choi KH, Lee SH, Song KS, Kang CS, Yu IJ: Aspect ratio has no effect on genotoxicity of multi-wall carbon nanotubes. *Arch Toxicol,* 85 (7): 775-786, 2011. DOI: 10.1007/s00204-010-0574-0

2. Ghaffri Chanzanagh E, Seifdavati J, Gheshlagh FMA, Benamar HA, Sharifi RS: Effect of ZnO nanoparticles on *in vitro* gas production of some animal and plant protein sources. *Kafkas Univ Vet Fak Derg,* 24 (1): 25-32, 2018. DOI: 10.9775/kvfd.2017.18187

3. Kılınç E: Fullerene C_{60} functionalized γ-Fe_2O_3 magnetic nanoparticle: Synthesis, characterization, and biomedical applications. *Artif Cells Nanomed Biotechnol,* 44 (1): 298-304, 2016. DOI: 10.3109/21691401.2014.948182

4. Pan X, Redding JE, Wiley PA, Wen L, McConnell JS, Zhang B: Mutagenicity evaluation of metal oxide nanoparticles by the bacterial reverse mutation assay. *Chemosphere,* 79 (1): 113-116, 2010. DOI: 10.1016/j.chemosphere.2009.12.056

5. Namdeo M, Saxena S, Tankhiwale R, Bajpai M, Mohan YM, Bajpai SK: Magnetic nanoparticles for drug delivery applications. *J Nanosci Nanotechnol,* 8 (7): 3247-3271, 2008. DOI: 10.1166/jnn.2008.399

6. Gupta AK, Gupta M: Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials,* 26 (18): 3995-4021, 2005. DOI: 10.1016/j.biomaterials.2004.10.012

7. Monteiro-Riviere NA, Nemanich RJ, Inman AO, Wang YY, Riviere JE: Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicol Lett,* 155 (3): 377-384, 2005. DOI: 10.1016/j.toxlet.2004.11.004

8. Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J: Evaluation of genotoxicity of multi-walled carbon nanotubes in a battery of *in vitro* and *in vivo* assays. *Regul Toxicol Pharmacol,* 63 (2): 188-195, 2012. DOI: 10.1016/j.yrtph.2012.03.014

9. Naya M, Kobayashi N, Endoh S, Maru J, Honda K, Ema M, Tanaka J, Fukumuro M, Hasegawa K, Nakajima M, Hayashi M, Nakanishi J: *In vivo* genotoxicity study of single-wall carbon nanotubes using comet assay following intratracheal instillation in rats. *Regul Toxicol Pharmacol,* 64 (1): 124-129, 2012. DOI: 10.1016/j.yrtph.2012.05.020

10. Ito A, Shinkai M, Honda H, Kobayashi T: Medical application of functionalized magnetic nanoparticles. *J Biosci Bioeng,* 100 (1): 1-11, 2005. DOI: 10.1263/jbb.100.1

11. Franchi LP, Manshian BB, de Souza TAJ, Soenen SJ, Matsubara EY, Rosolen JM, Takahashi CS: Cyto-and genotoxic effects of metallic nanoparticles in untransformed human fibroblast. *Toxicol In Vitro,* 29 (7): 1319-1331, 2015. DOI: 10.1016/j.tiv.2015.05.010

12. Co-operation OfE, Development: Test No. 471: Bacterial Reverse Mutation Test. OECD Publishing, 1997.

13. Osugi ME, Rajeshwar K, Ferraz ERA, de Oliveira DP, Araújo ÂR, Zanoni MVB: Comparison of oxidation efficiency of disperse dyes by chemical and photoelectrocatalytic chlorination and removal of mutagenic activity. *Electrochim Acta,* 54 (7): 2086-2093, 2009. DOI: 10.1016/j.electacta.2008.07.015

14. Devlin TM: Textbook of Biochemistry with Clinical Correlations. 2006.

15. Jarvis AS, Honeycutt ME, McFarland VA, Bulich AA, Bounds HC: A comparison of the Ames assay and Mutatox in assessing the mutagenic potential of contaminated dredged sediment. *Ecotoxicol Environ Saf,* 33 (2): 193-200, 1996. DOI: 10.1006/eesa.1996.0025

16. Kılınç E: γ-Fe_2O_3 magnetic nanoparticle functionalized with carboxylated multi walled carbon nanotube: Synthesis, characterization, analytical and biomedical application. *J Magn Magn Mater,* 401, 949-955, 2016. DOI: 10.1016/j.jmmm.2015.11.003

17. Ames BN, Lee FD, Durston WE: An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc Natl Acad Sci U S A,* 70 (3): 782-786, 1973. DOI: 10.1073/pnas.70.3.782

18. Maron DM, Ames BN: Revised methods for the *Salmonella* mutagenicity test. *Mutat Res,* 113, 173-215, 1983. DOI: 10.1016/0165-1161(83)90010-9

19. Di Sotto A, Chiaretti M, Carru GA, Bellucci S, Mazzanti G: Multi-walled carbon nanotubes: Lack of mutagenic activity in the bacterial reverse mutation assay. *Toxicol Lett,* 184 (3): 192-197, 2009. DOI: 10.1016/j.

toxlet.2008.11.007

20. Negi PS, Jayaprakash GK, Jena BS: Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chem,* 80, 393-397, 2003. DOI: 10.1016/S0308-8146(02)00279-0

21. Alexiou C, Jurgons R, Seliger C, Iro H: Medical applications of magnetic nanoparticles. *J Nanosci Nanotechnol,* 6, 2762-2768, 2006. DOI: 10.1166/jnn.2006.464

22. Szendi K, Varga C: Lack of genotoxicity of carbon nanotubes in a pilot study. *Anticancer Res,* 28, 349-352, 2008.

23. Tran C, Hankin SM, Ross B, Aitken RJ, Jones AD, Donaldson K, Stone V, Trantra R: An outline scoping study to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres. *Report on project CB0406*, 2008.

24. McShan D, Ray PC, Yu H: Molecular toxicity mechanism of nano-silver. *J Food Drug Anal,* 22 (1): 116-127, 2014. DOI: 10.1016/j.jfda.2014.01.010

25. Armentano I, Arciola CR, Fortunati E, Ferrari D, Mattioli S, Amoroso CF, Rizzo J, Kenny JM, Imbriani M, Visai L: The interaction of bacteria with engineered nanostructured polymeric materials: a review. *ScientificWorldJournal,* 2014:410423, 2014. DOI: 10.1155/2014/410423

26. Peters A, Veronesi B, Calderón-Garcidueñas L, Gehr P, Chen LC, Geiser M, Reed W, Rothen-Rutishauser B, Schürch S, Schulz H: Translocation and potential neurological effects of fine and ultrafine particles a critical update. *Part Fibre Toxicol,* 3:13, 2006. DOI: 10.1186/1743-8977-3-13

27. Geiser M, Rothen-Rutishauser B, Kapp N, Schürch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J, Gehr P: Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ Health Perspect,* 113 (11): 1555-1560, 2005. DOI: 10.1289/ehp.8006

28. Porter AE, Muller K, Skepper J, Midgley P, Welland M: Uptake of C_{60} by human monocyte macrophages, its localization and implications for toxicity: Studied by high resolution electron microscopy and electron tomography. *Acta Biomater,* 2 (4): 409-419, 2006. DOI: 10.1016/j.actbio.2006.02.006

29. Buzea C, Pacheco II, Robbie K: Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases,* 2 (4): MR17-MR71, 2007. DOI: 10.1116/1.2815690

30. Donaldson K, Poland CA: Nanotoxicity: Challenging the myth of nano-specific toxicity. *Curr Opin Biotechnol,* 24 (4): 724-734, 2013. DOI: 10.1016/j.copbio.2013.05.003

31. Schins RPF, Knaapen AM: Genotoxicity of poorly soluble particles. *Inhal Toxicol,* 19 (Suppl.1): 189-198, 2007. DOI: 10.1080/08958370701496202

32. Oberdörster G, Oberdörster E, Oberdörster J: Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect,* 113 (7): 823-839, 2005. DOI: 10.1289/ehp.7339

33. Brown DM, Donaldson K, Borm PJ, Schins RP, Dehnhardt M, Gilmour P, Jimenez LA, Stone V: Calcium and ROS-mediated activation of transcription factors and TNF-α cytokine gene expression in macrophages exposed to ultrafine particles. *Am J Physiol Lung Cell Mol Physiol,* 286 (2): L344-L353, 2004. DOI: 10.1152/ajplung.00139.2003

34. Jia G, Wang H, Yan L, Wang X, Pei R, Yan T, Zhao Y, Guo X: Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene. *Environ Sci Technol,* 39 (5): 1378-1383, 2005. DOI: 10.1021/es048729l

35. Bakry R, Vallant RM, Najam-ul-Haq M, Rainer M, Szabo Z, Huck CW, Bonn GK: Medicinal applications of fullerenes. *Int J Nanomedicine,* 2 (4): 639-649, 2007.

36. Partha R, Conyers JL: Biomedical applications of functionalized fullerene-based nanomaterials. *Int J Nanomedicine,* 4, 261-275, 2009. DOI: 10.2147/IJN.S5964

37. Mori T, Takada H, Ito S, Matsubayashi K, Miwa N, Sawaguchi T: Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. *Toxicology,* 225 (1): 48-54, 2006. DOI: 10.1016/j.tox.2006.05.001

38. Tsuchiya T, Yamakoshi YN, Miyata N: A novel promoting action of fullerene C_{60} on the chondrogenesis in rat embryonic limb bud cell culture system. *Biochem Biophys Res Commun,* 206 (3): 885-894, 1995. DOI: 10.1006/bbrc.1995.1126

39. Prylutska SV, Matyshevska OP, Golub AA, Prylutskyy YI, Potebnya GP, Ritter U, Scharff P: Study of C_{60} fullerenes and C_{60}-containing composites cytotoxicity in vitro. *Mater Sci Eng C Mater Biol Appl,* 27, 1121-1124, 2007. DOI: 10.1016/j.msec.2006.07.009

40. Shimizu K, Kubota R, Kobayashi N, Tahara M, Sugimoto N, Nishimura T, Ikarashi Y: Cytotoxic effects of hydroxylated fullerenes in three types of liver cells. *Materials,* 6 (7): 2713-2722, 2013. DOI: 10.3390/ma6072713

41. Shinohara N, Matsumoto K, Endoh S, Maru J, Nakanishi J: *In vitro* and *in vivo* genotoxicity tests on fullerene C_{60} nanoparticles. *Toxicol Lett,* 191 (2-3): 289-296, 2009. DOI: 10.1016/j.toxlet.2009.09.012

42. Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, Sayes CM: Development of a base set of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management. *Toxicol Lett,* 171 (3): 99-110, 2007. DOI: 10.1016/j.toxlet.2007.04.008