

# Mutagenicity Survey and Antibacterial Activity of Various Generations of Poly (Amid Amine) (PAMAM) Dendrimers and Algonac Acid Poly (Amid Amine) (PAMAM) Dendrimer Nano Composite G2 on Some Enteric Pathogenic Bacteria

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**Background:** Nano composites are widely used in medical sciences recently. Identification of chemical mutagens is an important issue in drug safety.

**Objectives:** The aim of this study was to investigate mutagenicity and antibacterial activity of various generations of poly (amid amine) (PAMAM) dendrimers and agonic acid poly (amid amine) (PAMAM) Nano composite G2 on enteric pathogenic bacteria.

**Materials and Methods:** Disc diffusion method, MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) determination were performed for antibacterial activity survey. Mutagenic properties were determined by Ames assay.

**Results:** Various dilutions of PAMAM generations (G2, G3, G4 and G5) and agonic acid PAMAM Nano composite G2 had antibacterial effect on *Escherichia coli*, *Salmonella enterica*, *Salmonella typhimurium*, *Enterococcus faecalis* and *Staphylococcus aureus*. In Ames assay, reverted colonies were increased by PAMAM generations increasing. Moreover, reverted colonies Ames assay were lower when Nano composite G2 was applied.

**Conclusions:** PAMAM generations and agonic acid PAMAM Nano composite G2 contain compounds with therapeutic potential and antibacterial characteristics, which can be used in medicine. Because some chemicals have mutagenic and carcinogenic properties, identification of these chemical is of great importance.

**Keywords:** Mutagenicity; Antibacterial; Enterobacteriaceae

## 1. Background

Algonac acid or alginate is an anionic polysaccharide found in the cell walls of brown algae. When mixing alginate with water, a viscous gum is formed. This substance absorbs water quite rapidly in extracted forms. It can water 200-300 times of its own weight. Pharmacological effects of agonic acid have been proved (1, 2). On the other hand, poly amid amine (PAMAM) dendrimers are nanoparticles used in medical sciences. Dendrimers are a class of hyper branched and Nano scale macromolecules. All these materials are originated from a nucleus and described as three-dimensional macromolecules with branching structures. They have regular structure and multiple functions in terminal groups and have spaces between the branches. These empty spaces can accept guest molecules, so encapsulation of particles in different sizes is possible. For this reason, scientists have used this material in the treatment of tumors, cancer cells and viral and bacterial infections (3-5). Today, using nanopar-

ticles in all aspects of human life is quickly progressing. Because of low toxicity of these substances, nanoparticles are used in medicine (6-9). Nano composite is a multiphase solid material, which one of its phases has one, two or three dimensions less than 100 nanometers (nm) in length (10). Antimicrobial activity of Nano composites has been proved (11). Identifying mutagen materials has become an important issue in drug safety. Therefore investigating mutagenicity of chemicals can be both valuable and useful in different aspects of medicine (12, 13). *Salmonella typhimurium* micro some assay (Ames test) is an accepted short-term bacterial assay or a biological test to detect mutagenic potential of chemical compounds and identifying substances that can produce genetic damage leading to gene mutation (14, 15).

## 2. Objectives

For these reasons, this study aimed to investigate antibacterial activity of various generations of poly (amid

amine)(PAMAM) dendrimers and agonic acid poly (amid amine) (PAMAM) Nano composite G2 on *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium*, *Enterococcus faecalis* and *Staphylococcus aureus* by disc diffusion, MIC (minimum inhibitory concentration) and MBC (minimum Bactericidal concentration) determination. Besides, to determine mutagenicity of these materials using the Ames test.

### 3. Materials and Methods

#### 3.1. Generation and Synthesis of Polyamide-Amine PAMAM

First, ethylene diamante ethylenediamine (10 g, 0.166 mol) was dissolved in 100 mL of methanol and then acetyl methyl acrylate (94.6 g, 0.751 mol) was added to the solution at 40°C. The solution was under nitrogen for 24 hours and then ethylenediamine (120 g, 2 mol) was added providing a whole generation. After repeating the above mentioned cycle generations 2, 3, 4 and 5 of PAMAM were prepared.

#### 3.2. Synthesis of Nano composite Alginate Dendrimer G2

First, sodium alginate was admixed with magnetic stirring in 1000 mL of distilled water for 24 hours at room temperature (27°C). The extra carboxyl groups within it were chlorinated by thionyl chloride, and then 1 mL of G2 dendrimer was added to the desired solution in strong acidic pH, so a complex between dendrimer NH<sup>3+</sup> groups and alginate COO<sup>-</sup> was built after 24 hours. Then this Nano composite was mixed with 1 mL of methanol solution and 2-chloroethyl isothiocyanate was added for 12 hours. Then dodecyl dimethyl amine was added to the mixture. Solution was heated at 80°C for 72 hours and placed at room temperature to get cold. The concentrated solution was purified with acetone. The purified solution was then filtered and dried in the oven. Then different concentrations were prepared.

#### 3.3. Bacterial Cultures

*E. coli* (ATCC 31150), *S. typhi* (PTCC 1609), *S. typhimurium* (ATCC 14028), *E. faecalis* (ATCC 29212) (Pasteur Institute, Iran) and *S. aureus* (ATCC 2592) (Difco, USA) were cultured in the form of linear culture on Muller-Hinton agar (MHA) medium (Merck, Germany) plates and then were incubated at 35°C for 24 hours. Re-confirmation of these bacteria was performed at laboratory of Microbiology Department, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran. Laboratory diagnoses such as gram staining, growth in Eosin-Methylene Blue (EMB) agar, coagulase, oxidase and catalase and IMViC tests, microscopic observation of gram negative and positive shapes were performed before initiation of examinations.

#### 3.4. Suspension Preparation

Fresh cultivated bacterial colonies were suspended in 5 mL of 0.85% normal saline. Suspension was mixed for 15 seconds with a vortex. Then its concentration was adjusted to 1.5 × 10<sup>8</sup> CFU/mL based on the standard 0.5 McFarland.

#### 3.5. Determining Antibacterial Activities Using the Disc Diffusion Method

Agar disc diffusion method was used to determine the antibacterial activity in this study. Suspension of bacteria (1.5 × 10<sup>8</sup> CFU/mL) through the lawn culture was planted on MHA plates by swap. Sterile blank discs with diameter of 6 mm (Pattan Teb, Iran) were impregnated with 0.1, 0.01, 0.001, 0.0001 dilutions of PAMAM generations (G2, G3, G4 and G5) and agonic acid PAMAM Nano composites G2. After drying, standard discs were put on agar media with sterile forceps inoculated by bacteria. Positive controls were gentamicin (30 µg) discs (Master Group Ltd, England) and negative control was a disc containing DMSO. Plates were incubated at 35°C for 24 hours. This step was performed according to the CLSI standard (This step was repeated for each bacterium and each dendrimer, separately) (16).

#### 3.6. MIC Determination

MIC determination was performed using the serial dilutions method. First, suspension of bacteria according to the standard 0.5 McFarland was provided in Müller-Hinton Broth (MHB) (Merck, Germany). Eleven tubes were considered for this phase. One millimeter of MHB was added to them. Next, 1 mL of agonic acid (PAMAM) G2 was added to tubes according to serial dilutions procedure. In addition, 20 µL of bacterial suspension was added to the tubes and then incubated at 35°C for 24 hours. Tube number 11 was considered as the control tube (1 mL MHB + 20 µL bacteria suspension). After incubation, MIC was determined by assessing turbidity. For PAMAM generations, this step was repeated (This step was repeated for each bacterium and each dendrimer, separately).

#### 3.7. MBC Determination

To determine MBC, tubes without turbidity were taken, and then cultured in MHA by swap. Plates were incubated at 35°C for 18 hours. MBC was considered as colony-forming unit (CFU), which is an estimate of viable or fungal numbers. Minimum concentration that inhibited the growth of bacteria was considered as MBC. Each step was repeated three times (This step was repeated for each bacterium and each dendrimer, separately) (17, 18).

#### 3.8. Ames Test, Media Preparation

Media used for the Ames test were Glucose minimum agar (10% glucose), Top agar with 0.005 mM Biotin-Histidine and nutrient broth (Merck company, Germany) (19).

### 3.9. Ames Test, Genotype of *S. Typhimurium*

*S. typhimurium* genotypes, strain TA100 was used for this study. The strain had histidine mutation in the histidine operon and this strain had additional mutations/genetic alterations, which made these strains more sensitive to chemicals. Strains used in this test were analyzed for their genetic integrity and spontaneous mutation rate when frozen cultures were prepared. A strain checking was performed when completing each experiment. It was performed by nutrient broth overnight cultures as follows: 1) Histidine dependence, 2) Biotin and histidine dependence, 3) rfa marker, 4) presence of plasmid PKM101 (Ampicillin resistance), 5) uvr B mutation (Table. 1)(19- 21).

## 4. Results

### 4.1. Results of Determining Antibacterial Activities Using the Disc Diffusion Method

Various generations of PAMAM dendrimers and agonic acid PAMAM Nano composite G2 on bacteria showed an antibacterial effect on *E. coli*, *S. typhi*, *S. typhimurium*, *E. faecalis* and *S. aureus*. In this study, by increasing the dilution, inhibition zone around the disc was increased and optimum sensitivity was observed.

### 4.2. Results of MIC and MBC Determination of Different Dilutions of Various Generations of PAMAM Dendrimers and Algonac Acid PAMAM Nano Composite G2

MIC and MBC of various generations of PAMAM were increased by generations increasing. For *E. coli*, optimum MIC (0.001) was related to G3, G4 and G5 and optimum

MBC (0.001) was related to G5. For Nano composite G2, effective MIC (0.001) was similar to G3, G4 and G5 MIC and effective MBC (0.01) was similar to MBC of G3 and G4. For *S. typhi*, MIC (0.001) in G4 and G5 and MBC (0.001) in G5 showed higher antimicrobial effect. Optimum MIC (0.001) of Nano composite G2 was similar to G4 and G5 MIC and its MBC (0.01) was similar to G4 MBC. Besides, for *S. typhimurium*, *E. faecalis* and *S. aureus*, optimum MIC (0.0001) was related to G4 and G5. *S. typhimurium* in G5 and also *E. faecalis* and *S. aureus* in G4 and G5 showed better MBC (0.001) than other generations. For *S. typhimurium* and *S. aureus*, more effective MIC (0.001) of nanocomposite G2 was similar to G2 and G3 MIC and for *E. faecalis*, optimum MIC (0.0001) of Nano composite G2 was similar to G4 and G5 MIC. Optimum Nano composite G2 MBC (0.01) for *S. typhimurium* was similar to G4 MBC and in *S. aureus* was similar to G2 and G3 MBC. Furthermore, more effective MBC of Nano composite G2 was 0.0001 for *E. faecalis*. Therefore, optimum inhibitory dilutions of generations of PAMAM and Alginic acid PAMAM Nano composite G2 were similar almost. Serial dilutions for MIC and MBC are shown in Tables 2 and 3.

### 4.3. Results of Ames Test

Inhibitory percentage of the agonic acid PAMAM Nano composites G2 used in the Ames test was 70.78%. Inhibitory percentage of the PAMAM generations used in the Ames test were 69.47%, 68.42%, 64.21% and 64.21% for

**Table 1.** Characteristics of *S. Typhimurium* Genotypes, Strain TA100 in This Study

Mutation (Strain)	Deletion Mutation	LPS Defect	Plasmid
his G46 (TA100)	bio-his uvr B	rfa	pKM101

**Table 2.** MIC Determination of Various Generations of PAMAM Dendrimers and Algonac Acid PAMAM Nano Composite G2 on Enteric Pathogenic Bacteria

Bacteria	PAMAM G2	PAMAM G3	PAMAM G4	PAMAM G5	Algonac acid PAMAM Nano composite G2
<i>E. coli</i>	0.01	0.001	0.001	0.001	0.001
<i>S. typhi</i>	0.01	0.01	0.001	0.001	0.001
<i>S. typhimurium</i>	0.001	0.001	0.0001	0.0001	0.001
<i>E. faecalis</i>	0.001	0.001	0.0001	0.0001	0.0001
<i>S. aureus</i>	0.001	0.001	0.0001	0.0001	0.001

**Table 3.** MBC Determination of Various Generations of PAMAM Dendrimers and Agonic Acid PAMAM Nano Composite G2 on Enteric Pathogenic Bacteria

Pathogenic Bacteria	PAMAM G2	PAMAM G3	PAMAM G4	PAMAM G5	Algonac Acid PAMAM Nano Composite G2
<i>E. coli</i>	0.1	0.01	0.01	0.001	0.01
<i>S. typhi</i>	0.1	0.1	0.01	0.001	0.01
<i>S. typhimurium</i>	0.1	0.1	0.01	0.001	0.01
<i>E. faecalis</i>	0.01	0.01	0.001	0.001	0.0001
<i>S. aureus</i>	0.01	0.01	0.001	0.001	0.01

the G2, G3, G4 and G5, respectively. Numbers of reverted colonies were increased by generations increasing and significant differences were observed. Reverted colonies were lower when Nano composites G2 was used instead of PAMAM generations in Ames test.

#### 4.4. Results of Statistical Analysis

ANOVA result showed significant difference for all returned colonies. The maximum difference between the mean of returned colonies was related to sterile distilled water and sodium aside with six colonies and P value of 0.001. Between different generations of PAMAM, the least difference was related to PAMAM-G2, G3, G4 and G5 compared to sterile distilled water and DMSO regarding the number of returned colonies, respectively. P value for PAMAM-G2 was 0.014 and the mean of returned colonies was 65.95. Moreover, different generations of PAMAM showed no significant difference. However, Kruskal-Wallis test showed significant difference between sodium aside and other tested materials. A significant difference was shown between test and control groups ( $P < 0.05$ ). Ames assay performed on water, sodium aside and agonic acid PAMAM Nan Composites G2 are shown in Figures 1-3.

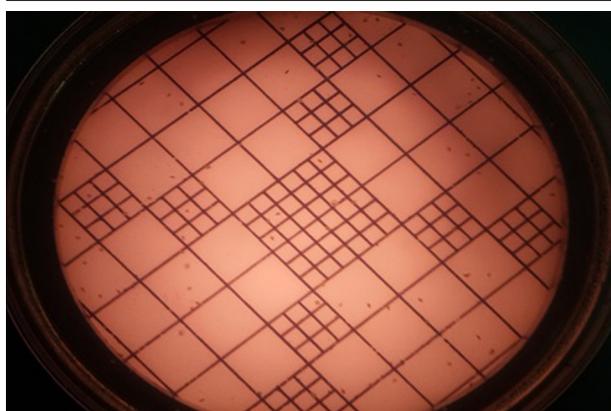


Figure 1. Ames Assay Result on Water as Negative Control



Figure 2. Ames Assay Result on Sodium Aside as Positive Control

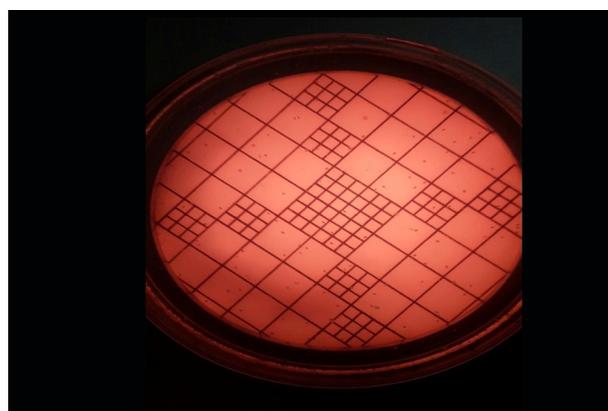


Figure 3. Ames Assay Result on Algonic acid PAMAM Nano Composites G2

## 5. Discussion

Dendrimers Nano composites are organic-inorganic hybrid nanoparticles synthesized by dispersing very small inorganic domains in nanoscopic polymeric networks. They are used for biomedical applications. Our results showed that various generations of PAMAM and agonic acid PAMAM Nano composites G2 had antibacterial effect on *E. coli*, *S. typhi*, *S. typhimurium*, *E. faecalis* and *S. aureus*. Researchers have shown application of dendrimers, dendritic and different Nano composite structures in medicine (11, 20, 21). Palomba et al. showed that silver polystyrene Nano composites had antimicrobial activity against *E. coli* (11). In another study Saadatmand et al. from Iran showed antimicrobial properties of chitosan-TiO<sub>2</sub> Nano composite against *E. coli* and *S. aureus* and also its usage on sterile gauze pads (22). In our study, PAMAM Nano composites G2 (MIC: 0.001, MBC: 0.01) showed antibacterial effects against *E. coli*. Nanoparticles have antibacterial activities and different electronic, optical, mechanical, magnetic, and chemical properties. Nevertheless silver polystyrene Nano composites showed antibacterial activities against *E. coli* in a similar way to our study. Valipour et al. from Iran showed that Nano composites containing ZnO and TiO<sub>2</sub> nanoparticles exhibit antibacterial activity against *E. coli* and *S. aureus* (23). In addition, our results indicated that Algonic acid PAMAM Nano composite G2 had antibacterial activity against *E. coli*, *S. typhi*, *S. typhimurium*, *E. faecalis* and *S. aureus*. Winnicka et al. in Poland showed that PAMAM dendrimers G2 have antibacterial activity against *S. aureus* (24). Xue et al. in China reported that amino-terminated PAMAM G2 has antibacterial activity. In our study, antibacterial properties of PAMAM generations were shown.

Antibacterial mechanism of PAMAM is unknown, but several studies have proved that its antibacterial activity is related to PAMAM generation and G2 has better antibacterial activity (25). Cytoplasmic membrane of bacterial cell is a target for many antimicrobial agents. In addition, different studies have shown that hydroxyl and

carboxylic acid terminated PAMAM dendrimers showed antibactericidal activities on bacterial cell wall. Therefore, they can penetrate bacterial cell and release intracellular compounds and eventually destroying the bacteria cell (24, 26). Considering increased antibacterial drug resistance, these chemicals can be used as antibacterial agents. Nonetheless, different studies have shown mutagenicity of different chemicals by the Ames test. Shahhosseiny et al. in Iran using the Ames test on *S. typhimurium* TA98 and TA100 reported the presence of mutagenic substances in Tehran's air pollutants (27). In another study, Partoazar et al. in Iran investigating 57 urine samples of medicine laboratory personals using the Ames test by *S. typhimurium* (TA98) showed that these samples caused mutagenicity (28). However, some studies showed that PAMAM dendrimers have genotoxicity effects on cells (25, 29). In our study, Ames test showed that different generations of PAMAM (G2, G3, G4 and G5) had mutagenicity effects and reverted colonies were increased by higher-generation of PAMAM dendrimers. The Ames test has specific features such as sensitivity and specificity; so different materials can be used in this test (30). Pounikar and Dawande using the Ames test showed that ethidium bromide, sodium aside, hair dye, colors and food additives had mutagenic response on auxotrophic strain (his-) and induced reversion mutation (his+). Results of Ames test in this study showed that inhibition percent of G2, G3, G4, and G5 were 69.47%, 68.42%, 64.210% and 64.21%, respectively. Different materials used in Ames assay may produce a reproducible dosage and it increases the number of reverting colonies in strains of *S. typhimurium* (31). Akyil and Konuk in Turkey showed pesticide mutagenicity by the Ames test on TA97, TA100, and TA102 strains of *S. typhimurium* (32). *S. typhimurium* strain TA100 was used for our study, because Salmonella strains have different mutations in various genes of histidine operon. Salmonella mutagenicity test is designed to detect chemicals able to induce mutagenesis. Application of PAMAM in Nano medicine is increased, but taking into account the toxicity of PAMAM, usage of these materials should be with caution. In our study, it was shown that reverted colonies were lower when Nano composite G2 was applied in the Ames test, so it is a better and safer antibacterial substance compared to PAMAM generations. In conclusion, safety of these materials must be considered before applying them and also toxicity of these compounds should be evaluated by in-vivo and in-vitro studies.

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## Authors' Contributions

All authors participated in preparing the manuscript and experiment procedures equally.

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## References

- Rowe H, McCallum S, Le MT, Vittorino R. Admission to day stay early parenting program is associated with improvements in mental health and infant behaviour: A prospective cohort study. *Int J Ment Health Syst.* 2012;**6**(1):11.
- Hampson FC, Farndale A, Strugala V, Sykes J, Jolliffe IG, Dettmar PW. Alginate rafts and their characterisation. *Int J Pharm.* 2005;**294**(1-2):137-47.
- Baigude H, Katsuraya K, Okuyama K, Uryu T. Synthesis of Structurally-Controlled AIDS Vaccine Model with Glyco-Peptide Dendrimer Scaffolds. *Macromol Chem Phys.* 2004;**205**(5):684-91.
- Duan X, Sheardown H. Dendrimer crosslinked collagen as a corneal tissue engineering scaffold: mechanical properties and corneal epithelial cell interactions. *Biomaterials.* 2006;**27**(26):4608-17.
- Hong MY, Lee D, Yoon HC, Kim HS. Patterning Biological Molecules onto Poly(amidoamine) Dendrimer on Gold and Glass. *Korean Chem Soc.* 2003;**24**(8):1197-202.
- Hatchett DW, Josowicz M. Composites of intrinsically conducting polymers as sensing nanomaterials. *Chem Rev.* 2008;**108**(2):746-69.
- Kumar A, Jakhmola A. RNA-mediated fluorescent Q-PbS nanoparticles. *Langmuir.* 2007;**23**(6):2915-8.
- Aslan K, Gryczynski I, Malicka J, Matveeva E, Lakowicz JR, Geddes CD. Metal-enhanced fluorescence: an emerging tool in biotechnology. *Curr Opin Biotechnol.* 2005;**16**(1):55-62.
- Sosa IO, Noguez C, Barrera RG. Optical Properties of Metal Nanoparticles with Arbitrary Shapes. *Phys Chemist B J.* 2003;**107**(26):6269-75.
- Ajayan PM, Schadler S, Braun PV. Nanocomposite science and technology. *Wiley int J.* 2003;**1**(1).
- Palomba M, Carotenuto G, Cristino L, Di Grazia MA, Nicolais F, De Nicola S. Activity of Antimicrobial Silver Polystyrene Nanocomposites. *Nanomat int J.* 2012;**2012**:1-7.
- Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. *Food Chem Toxicol.* 2002;**40**(8):1091-7.
- Zegura B, Dobnik D, Niderl MH, Filipic M. Antioxidant and antigenotoxic effects of rosemary (*Rosmarinus officinalis* L.) extracts in Salmonella typhimurium TA98 and HepG2 cells. *Environ Toxicol Pharmacol.* 2011;**32**(2):296-305.
- Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. *Mutat Res.* 2000;**455**(1-2):29-60.
- Thakkar Jalaram H, Patel Chirag A, Santani Devdas D, Jani Girish K. Evaluation of Invitro antimutagenic potential of lagenaria siceraria using Ame's test. *Am J Cancer Biol.* 2013;**1**(2):2-5.
- Clinical and Laboratory Standards Institute (CLSI). performance standards for antimicrobial susceptibility testing twentieth informational supplement m100-s20, M02-A10: Performance standards for antimicrobial disk susceptibility tests. *approv stand J.* 2010:1-153.
- Kretschmar M, Hube B, Bertsch T, Sanglard D, Merker R, Schroder M, et al. Germ tubes and proteinase activity contribute to virulence of *Candida albicans* in murine peritonitis. *Infect Immun.* 1999;**67**(12):6637-42.
- Kumar SS, Kamaraj M. Analysis of phytochemical constituents antimicrobial activities of *Cucumis angur*. L. against clinical pthogene. *American-Eurasian J Agric Environ Sci.* 2010;**7**(7-8):176-8.
- Rezaei-Basiri M, Rezazadeh H, Aswadi-Kermani I, Ghazi-Khansari M. Anti-mutagenicity Effects of Vitamin E on Oncology and Non-oncology Hospital Nurses by Ames Assay. *J Clin Diagn Res.* 2013;**7**(12):2917-21.
- Wolinsky JB, Grinstaff MW. Therapeutic and diagnostic applications of dendrimers for cancer treatment. *Adv Drug Deliv Rev.* 2008;**60**(9):1037-55.

21. Silva JRNP, Menacho FP, Chorilli M. Dendrimers as potential platform in nanotechnology-based drug delivery systems. *J Pharm.* 2012;**2**(5):23–30.
22. Saadatmand MM, Yazdanshenas ME, Rezaei-Zarchi S, Yousef-telori B, Negahdary ML. [Investigation of anti-microbial properties of chitosan-Tio2 nanocomposite and its use on sterile gauze pads.]. *lab Sci.* 2012;**6**(1):59–79.
23. Valipour P, Seyedi L, Moosavian M. T. . [Preparation and evaluation of antibacterial polypropylene film using nano-coatig containing zinc oxide and titanium dioxide nanoparticles]. *J Tjoe-textile Sci Technol.* 2012;**2**(2):59–63.
24. Winnicka K, Wroblewska M, Wiczorek P, Sacha PT, Tryniszewska EA. The effect of PAMAM dendrimers on the antibacterial activity of antibiotics with different water solubility. *Molecules.* 2013;**18**(7):8607–17.
25. Xue X, Chen X, Mao X, Hou Z, Zhou Y, Bai H, et al. Amino-terminated generation 2 poly(amidoamine) dendrimer as a potential broad-spectrum, nonresistance-inducing antibacterial agent. *AAPS J.* 2013;**15**(1):132–42.
26. Wang B, Navath RS, Menjoge AR, Balakrishnan B, Bellair R, Dai H, et al. Inhibition of bacterial growth and intramniotic infection in a guinea pig model of chorioamnionitis using PAMAM dendrimers. *Int J Pharm.* 2010;**395**(1-2):298–308.
27. Shahhosseiny MH, Bagheri MJ, SoltanDallal MM, KhoramKhorshid MR. [Assessment of mutagenicity and carcinogenicity of Tehran's air pollutants with Ames test]. *Hakim Res J.* 2008;**11**(1):29–39.
28. Partoazar A, Ghazi Khansari M, Abedi MH, Kaviani M, Norashrafeddin SM, Basiri MR. Determining urine sample mutagenicity ratio using Ames test: Tehran forensic medicine laboratory personnel. *Tehran University Med J.* 2009;**67**(3):184–9.
29. Choi YJ, Kang SJ, Kim YJ, Lim YB, Chung HW. Comparative studies on the genotoxicity and cytotoxicity of polymeric gene carriers polyethylenimine (PEI) and polyamidoamine (PAMAM) dendrimer in Jurkat T-cells. *Drug Chem Toxicol.* 2010;**33**(4):357–66.
30. Welbel SF, Schoendorf K, Bland LA, Arduino MJ, Groves C, Schable B, et al. An outbreak of gram-negative bloodstream infections in chronic hemodialysis patients. *Am J Nephrol.* 1995;**15**(1):1–4.
31. Pounikar R, Dawande AY. Detection of potential carcinogens by Ames test. *Asiatic J Biotech Res.* 2010;**1**(1):57–64.
32. Akyil D, Konuk M. Detection of genotoxicity and mutagenicity of chlorthiophos using micronucleus, chromosome aberration, sister chromatid exchange, and Ames tests. *Environ Toxicol.* 2014.