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Genotoxicity and apoptotic activity of biologically synthesized magnesium oxide nanoparticles against human lung cancer A-549 cell line

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Abstract

The study focussed on the synthesis of magnesium oxide (MgO) nanoparticles from an aqueous extract of Penicillium species isolated from soil. A suitable amount of magnesium nitrate (MgNO₃) was mixed with the aqueous extract of Penicillium. Then the colour of the solution changed due to the formation of MgO nanoparticles. These nascent formed MgO nanoparticles were further confirmed by using UV spectrophotometry which showed the maximum absorption (λ_{max}) at 215 nm indicating the formation of MgO nanoparticles. Fourier transform infrared spectroscopy (FTIR) was used to find the possible functional groups and proteins involving the stabilization of MgO nanoparticles. Transmission electron microscopy (TEM) study revealed the size, the shape as well as the dispersity of the prepared MgO nanoparticles and showed that they were well dispersed around 12-24 nm (scale 200 nm). The anticancer activity against A-549 cell line of these green synthesized MgO nanoparticles was evaluated. The result showed good anticancer effect after 24h of incubation. Nevertheless these MgO nanoparticles showed less effect on normal Vero cells. Further apoptotic study clearly displayed the effect of MgO nanoparticles on cancer cells. The effect was observed through chromatin condensation by forming apoptotic bodies using propidium iodide, acridine orange and ethidium bromide (AO/EB) staining technique. The DNA was isolated to confirm the DNA damage; the observation clearly showed DNA damage when compared with DNA ladder.

Keywords: magnessium oxide nanoparticles, *Penicillium* sp., TEM, FTIR, apoptotic activity, anticancer activity Classification numbers: 2.03, 4.02, 5.08

1. Introduction

Cancer, a very dreadful disease characterized by the uncontrolled and abnormal growth of cells, was mostly treated by using radiation therapy, surgery and chemotherapy [1]. These therapies

Original content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI. are very good in destroying the uncontrolled growth of cancer cells, but at the same time they destroy also the normal cells [2]. Nowadays above-mentioned therapies are getting outdated due to the advancement in the field of nanobiotechnology and nanomedicine with specific targeted drug delivery which can inhibit the cells at different target sites [3]. Nanobiotechnology is the area in which nanoparticles are engineered for its biological and biomedical application. Nanotechnology has immense potential for therapeutic and imaging for early tumour detection



Figure 1. Colour changes of cell-free extract of Penicillium sp. before and after addition $Mg(NO_3)^{2-}$ biosynthesis of MgO nanoparticles where (A)—before addition of MgNO₃; (B)—after addition of AgNO₃—colour change.

and effective treatment of various cancers [4]. Nowadays nanotechnology especially nanomedicine showed the application in personalized medicine and for the detection of various cardia vascular disease [5, 6].

Nowadays various nanomaterials are synthesized like ZnO, MnO and MgO, which have immense potential in an increase in image contrast, can be used for diagnosis of diseases [7, 8]. Among various synthesized nanoparticles the CaO, ZnO and MgO nanoparticles are highly interested, because they are extremely stable in very harsh conditions [9, 10]. Magnesium oxide has large bandwidth with a wide range of applications, especially in toxic waste treatment, catalysis, anti-reflecting and reflecting coating, adsorbents, refractory materials [11, 12]. MgO nanoparticles showed good antibacterial and anticancer activities [13] and can be used for the treatment of heartburn, stomach sore and bone regeneration [14, 15].

Various approaches were used for the synthesis of nanoparticles like physical, chemical and biological methods, but the method using biological living organisms as biofactories like plants, bacteria, and fungi has the advantage because it is eco-friendly, cost-effective, easily available and straightforward [16–18].

This study aims to perform the biological synthesis of MgO nanoparticles from an aqueous extract of *Penicillium* species (*Penicillium* sp.), to characterize synthesized MgO nanoparticles by various microscopic techniques and to investigate the effect of these nanoparticles on A-549 cell line. The apoptotic activity of the cells and the DNA damage are also studied by using different stains PI (propidium iodide), AO, EB and gel electrophoresis.

2. Material and methods

2.1. Sample collection

The soil sample was collected from Universiti Kuala Lumpur Royal College of Medicine Perak (UniKL RCMP). The sample was transferred to sterile petri plate. Then, the plate was covered and was taken to Research Laboratory of UniKL RCMP and incubated at room temperature for drying purpose for further process.

2.2. Isolation of fungal culture

Serial dilution technique and spread plate method were used for the isolation of fungal culture. One gram of dried soil



Figure 2. UV–vis spectra recorded lambda max of MgO nanoparticles synthesized from *Penicillium sp.* is 215.49 nm.

sample was diluted in 10 ml of sterilized distilled water. Then, the dilution was serially diluted to prepare a dilution with concentration range from 10^{-1} ml until 10^{-5} ml. 0.5 ml volume of dilution from the concentration range of 10^{-3} ml until 10^{-5} ml was transferred aseptically and spread on the potato dextrose agar (PDA) plates respectively. The plates were placed in dark condition and incubated at room temperature for three days. To prepare a pure culture of *Penicillium* sp. the isolated fungi were sub cultured on the Sabouraud Dextrose Agar (SDA) plates which can identify by the morphology. The purely isolated *Penicillium* sp. was maintained at 4 °C for further studies.

2.3. Identification of Penicillium species

Penicillium sp. was observed by colony morphology concerning its size, colour, shape and nature of colony. The observation was conducted through microscopically and method was compared with available literature in the laboratory.

2.4. Biosynthesis of magnesium oxide nanoparticles

The purely isolated *Penicillium sp.* was employed for the synthesis of magnesium nanoparticle through extracellular method. Fungal biomass of *Penicillium* sp. was grown aerobically by adding the fungal spore in PDA broth and incubated at 25 °C on rotary shaker fixed at 160 rpm for 72 h. After 72 h, the fungal biomass was filtered by using Whatman filter paper No.1 and washed two to three times by using distilled water to remove the residual part. The fungal biomass was put into



Figure 3. Fourier transform infrared (FTIR) analysis recorded of MgO nanoparticles synthesize from Penicillium sp.

a conical flask containing 100 mL of distilled water and incubated at 25 °C on rotary shaker fixed at 160 rpm for next 72 h. After 72 h of incubation on the rotary shaker, the biomass was filtered by using Whatman filter paper No.1. The fresh, clean, clear and cell-free extract was taken into the clean conical flask for further study purpose. A suitable amount of magnesium nitrate (MgNO₃) was added into the conical flask and kept at 25 °C on rotary shaker fixed at 160 rpm for 72 h in the dark condition. The colour and appearance change during storage in dark room was observed.

2.5. Characterization of MgO nanoparticles

The colour change of solution was observed by optical appearance after the addition of MgNO₃ and was further confirmed by UV–vis spectrophotometric analyser with the wavelength of 300 nm to 600 nm to find the wavelength of maximum absorbance (λ_{max}). FTIR spectroscopy analysis has confirmed the essential functional groups and proteins which plays a significant role in stabilization of MgO nanoparticle. The powder form of nanoparticles was mixed with potassium bromide (KBr) to form a solid mixture of transparent film by using hydraulic presser and put in holder and subject for FTIR spectroscopy analysis. The MgO nanoparticles were centrifuged at 20000 rpm for 20 min, then supernatant of liquid was drained out and dried solid was converted into pellet was analyzed for structure through TEM analysis.

2.6. Antitumor activity of MgO nanoparticles

2.6.1. Cell line and cell culture. The alveolar lung cancer cell A-549 and Vero cell were obtained from the National center of cell science (NCCS) Poona India. Both cell lines were classicaly grown in MEM as monolayer supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) glutamine, 100 units ml^{-1} streptomycin, 100 units ml^{-1} penicillin at 37 °C under 5% carbon dioxide environment.

2.6.2. In vitro assay of cell viability. Biologically synthesized MgO nanoparticles were evaluated for its toxicity on normal Vero and A-549 lung cancer cell lines by MTT assay. The MgO

nanoparticles were dissolved in double distilled water (ddH₂O) and different concentration of nanoparticles were used such as $60 \,\mu \text{g}\,\text{ml}^{-1}$, $80 \,\mu \text{g}\,\text{ml}^{-1}$, $100 \,\mu \text{g}\,\text{ml}^{-1}$, $120 \,\mu \text{g}\,\text{ml}^{-1}$, $140 \,\mu \text{g ml}^{-1}$ and $160 \,\mu \text{g ml}^{-1}$ on cell culture grown on 96-well microplates and incubate for 24 h at 37 °C. After the incubation cells were washed in phosphate buffer solution followed by 100 ml of 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide, tetrazolium MTT solution was added properly to all 96 wells and kept for incubation for 2–3 h at 37 °C. 100 μ l dimethyl sulphoxide (DMSO) was added to each 96-wells microplate to solubilize the crystals formed by using MTT dye and after put in the shaker for 10-15 min so that all crystals of Vero and cancer cells will solubilize properly observed by producing the color. The viability of all cells was measured by using spectrophotometric analysis at 570nm. Viability of cells was measured by using the following relation

$$Percentage of Cell viability = \frac{Optical density (OD) of MgO nanoparticles}{Optical density (OD) of Control} \times 100.$$
(1)

The toxicity of MgO nanoparticles on cancer and normal vero cells was expressed the amount of drug that kills 50% of cells which can compared with the 50% of untreated cells as IC50 value. This biological experiment was done in triplicate and DMSO was kept as negative control.

2.6.3. Analysis of apoptotic activity by PI/AO/EB dual staining. The propidium iodide (PI), acridine orange (AO) and ethidium bromide (EB) staining was used analyze the morphological changes induced by MgO nanoparticles in human alveoli lung cancer A-549 cell line. For that A-549 cells were separately plated at $5 \cdot 10^4$ cells/well into a 6-well chamber plate. At >90% confluence and cells were treated with particular concentration of nanoparticles and incubate for 24 h. After incubation cells were washed with phosphate buffer saline (PBS) fixed in methanol: acetic acid (3:1, v/v)for 10 min and stained with $50 \,\mu g \, m l^{-1}$ of PI for 20 min. For determining the mode of cell death, the cells were seeded in seeded in 6-well plates and treated with inhibitory concentration (IC50) of nanoparticles. To determine the nuclear properties, the monolayer of cells was washed with the PBS and stained with 5 μ l of AO (100 μ g ml⁻¹) and 5 μ l of EB



Figure 4. Transmission electron microscopy (TEM) of MgO nanoparticles synthesized from *Penicillium sp.* with size range of (12 nm– 24 nm). where (A)–(C) are the magnification of samples at different angles while (D) is the magnification at 200 nm scale.



Figure 5. Cytotoxic effect MgO nanoparticles on A549 cancer cells; where (A)—control A549 cancer cells, (B)—after incubated with MgONPs—Morphological changes in the cells showing membrane damage.

 $(100 \,\mu \text{g ml}^{-1})$. The morphological and topological changes in the stained cells and their apoptotic nuclei were observed by fluorescent microscopy.

2.6.4. Analysis of DNA fragmentation. To determine the actual effect of nanoparticles on the DNA of A-549 (1×106 cells ml⁻¹) cancer cells were plated per well in 6-well plates with the Dulbecco's modified eagle medium (DMEM) contained 10% FBS. The cells were then incubated for 24 h under 5% CO₂ at 37 °C. Then the medium was removed, washed with PBS; fresh serum free medium was added and kept in a CO₂ incubator at 37 °C for 1 h.

2.6.5. Isolation of DNA. The starved cells were washed with PBS and then add 1 ml of lysis buffer mix properly and put in centrifuge tube. The whole mixture was incubated for 1 h at 37 °C and after incubation again add 5 μ l of proteinase K incubated at 50 °C for 3 h. After incubation add pheno/choloroform isopropyl alcohol (25:24:1). The aqueous layer was gently transferred to the new Eppendorf tube and the absolute chilled alcohol (ethanol) was added and mix along with stand followed by centrifugation at 13000 rpm for 10 min. After centrifugation the supernatant was discarded and the pellet was detached and treated 70% ethanol to remove the impurities.



Figure 6. Graphical representation of cytotoxic effect MgO nanoparticles on Cancer cells.

The sample was centrifuged at 11500 rpm for 5 min so that all traces of impurities should be removed. The supernatant was discarded and the pellet was dried for 2 h at 37 °C temperature and resuspended in 50 μ l of Tris EDTA (TE) buffer. 10 μ g ml⁻¹ of DNA samples were electrophoresed in 1% agarose gel containing ethidium bromide in a gel tank containing Tris-Borate EDTA (TBE) buffer for 1 h under 90 V. The gels were examined under a UV transilluminator.

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Figure 7. Cytotoxic effect of MgO nanoparticles on normal Vero cell line—where (A)—normal vero cells, (B)—after incubation with MgO nanoparticles.

3. Results and discussion

The aqueous extract of *Penicillium* sp. was utilized for the synthesis of MgO nanoparticles. When the cell filtrate was challenged with magnesium nitrate (MgNO₃) the color of solution becomes white cloudy indicates the synthesis of nanoparticles (figure 1). These MgO nanoparticles were characterized by UV–vis spectroscopy which showed the absorption peak at 215 nm confirms the presence of MgO nanoparticles due to surface plasmon vibration [19, 20] (figure 2).

FTIR spectroscopy was used to check and identify the biomolecules, functional groups and proteins which act as capping and play the main role in the stabilization of MgO nanoparticles using the aqueous extract of fungal species. The FTIR spectroscopy analysis showed the sharp absorption bands at 3508.22 cm^{-1} (OH-stretch phenols), 2770.03 cm^{-1} (C–H stretch aldehydes), 2428.57 cm^{-1} (C=N stretch), 1763.45 cm^{-1} (C=O stretch), 1624.78 cm^{-1} (N–H amines), 1560.29 cm^{-1} (N–O asymmetric stretch nitro), 1387.84 cm^{-1} (N–H alkanes), 1012.07 cm^{-1} (C–H alkanes), 827.11 cm^{-1} (C–H alkenes), 708.24 cm^{-1} (C–H alkanes) [21] (figure 3).

A high-resolution TEM was used to determine the size, shape and dispersity of MgO nanoparticles. The TEM analysis showed that MgO nanoparticles look spherical, properly dispersed and the size of nanoparticles is in the range of 12–24 nm (figure 4).

The cytotoxic effect of biologically synthesized MgO nanoparticles on A-549 human lung cancer cell line showed the excellent cytotoxic effect on the cancer cells that can be observed by morphological changes in the cell membrane by using various concentration of MgO nanoparticles. The results showed the IC50 value at $100 \,\mu g \, ml^{-1}$ upon 24 h of incubation which can be visually observed by cell membrane damage on a dose dependent manner [22, 23] (as shown in figure 5). Moreover Vero cell line incubated with MgO nanoparticles showed the IC50 value at 140 μ g ml⁻¹ which it showed less toxicity towards normal cells (figure 6). The change in morphology of the cells can be observed by the shrinking, and nonadherence to the surface due to the release of reactive oxygen species (ROS) which affected the essential enzymes in the cell by producing the oxidative distress that results in the destruction of the DNA inside the cell [24]. The vero cell line affected by the higher concentration of MgO nanoparticles when compared with dosage on cancer cells that means it has less effect towards normal cells (figure 7). Figures 6 and 8 show the





Figure 8. Graphical representation of cytotoxic effect MgO nanoparticles on normal Vero cell line.

graphical representation of the cytotoxic effect of MgO nanoparticles on A-549 cancer cell line and also Vero cell line.

Apoptotic activity of MgO nanoparticles was revealed by PI on A-549 cells to determine the nuclear morphology confirmed by membrane permeable PI stain. The treated cells contain more apoptotic cells when compare with untreated cells and damage of cells was observed by using 100 μ g ml⁻¹ can be change in size, decreased fluorescence confirming condensed nuclear chromatin in apoptotic cells and formation of membrane blebs.

Acridine orange and ethidium bromide (AO/EB) dual staining can show the overall morphological change in shape and size to the treated cell when compare with non-treated cells [25, 26]. The viable cell can give green colour which means the DNA is intact. Early apoptotic cells will have fragmented DNA which gives several green coloured nuclei. The DNA of late apoptotic and necrotic cells would be fragmented and stained orange and red. From the above data it is clear increase the concentration of nanoparticles increase the DNA damage and number of the viable cell will decrease which can be seen with less viable cells with more apoptotic cells (figure 9).

Analysis of DNA fragmentation was carried out on the A-549 lung cancer cells treated with MgO nanoparticles to induce the apoptosis of the cells. The DNA fragmentation and the damage of DNA can be analyzed through the death of cancer cells [27]. In the current study the DNA was damaged consequently no band was observed when compare with DNA ladder. Different kinds of DNA fragments from cancer cells

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Figure 9. AO/EB and PI staining of human alveoli A-549 cancer cells. where (A)—normal green colour live cell, (B)—orange colour cells are apoptotic bodies induced by MgO nanoparticles (C)—chromatin condensation induced by MgO nanoparticles.



Figure 10. DNA Fragmentation analysis against A549 cancer cells where L 4—Normal DNA ladder (control), L3—DNA of treated cells showing complete damage of DNA.

treated with MgO nanoparticles showed the DNA damage and hence no visualization of bands on the agarose gel electrophoresis was seen (figure 10).

4. Conclusions

In this study *Penicillium* species was first time used for biosynthesis of MgO nanoparticles. The TEM analysis showed the stability and nanosize of MgO nanoparticles and these MgO nanoparticles showed excellent anticancer activity and less effect towards normal cells. From this study it was showed how nanoparticles induced apoptotic activity and also DNA damage when treated with MgO nanoparticles. These MgO nanoparticles could be a good anticancer agent but needs further cytotoxicity study on animals before using as strong anticancer agent.

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Conflict of interest

There is no conflict of interest among authors.

References

- [1] Smalley K S and Herlyn M 2006 Mini Rev. Med. Chem. 6 387
- [2] Langer R 1998 *Nature* **392** 5
- [3] Gowda R, Jones N R, Banerjee S and Robertson G P 2013 J Nanomed. Nanotechnol. 4 6
- [4] Wang R, Billone P S and Mullett W M 2013 J. Nanomater. 2013 629681
- [5] Vizirianakis I S 2011 Nanomedicine 7 11
- [6] Godin B, Sakamoto J H, Serda R E, Grattoni A, Bouamrani A and Ferrari M 2010 Trends Pharmacol. Sci. 31 199
- [7] Na H B, Lee L H, An K, Park Y I, Park M, Lee I S, Nam D H, Kim S T, Kim S H and Kim S 2007 Angew. Chem., Int. Ed. 119 5493
- [8] Krishnamoorthy K, Moon J Y, Hyun H B, Cho S K and Kim S J 2012 J. Mater. Chem. 22 24610
- [9] Sundrarajan M, Suresh J and Gandhi R R 2012 Dig. J. Nano-Mater. Biostruct. 7 983
- [10] Stoimenov P K, Klinger R L, Marchin G L and Klabunde K J 2002 Langmuir 18 6679
- [11] Mirzaei H and Davoodnia A 2012 Chin. J. Catal. 33 1502
- [12] Ouraipryvan P, Sreethawong T and Chavadej S 2009 Mater. Lett. 63 1862
- [13] Di D R, He Z Z, Sun Z Q and Liu J 2012 *Nanomedicine* **8** 1233
- [14] Boubeta C M et al 2010 Nanomedicine 6 362
- [15] Bertinetti L, Drouet C, Combes C, Rey C, Tampieri A, Coluccia S and Martra G 2009 Langmuir 25 5647
- [16] Iravani S 2011 Green Chem. 13 2638

- [17] Thakkar K N, Mhatre S S and Parikh R Y 2010 Nanotechnol. Biol. Med. Nanomed. 6 257
- [18] Ebraminezhad A 2016 Adv. Nat. Sci.: Nanosci. Nanotechnol. 7 015018
- [19] Philip D 2008 Spectrochim. Acta A 71 80
- [20] Shankar S S, Rai A, Ahmad A and Sastry M 2004 J. Colloid Interface Sci. 275 496
- [21] Kowalski R, Wawrzykowski J and Zawislak G 2007 Herba Pol. **53** 246
- [22] Piao M J, Kang K A, Lee I K, Kim H S, Kim S, Choi J Y, Choi J and Hyun J W 2011 Toxicol. Lett. 201 92
- [23] Rani P V, Mun G L, Hande M P and Valiyaveettil S 2009 ACS Nano 3 279
- [24] Sriram M I, Kanth S M, Kalishwaralal K and Gurunathan S 2010 Int. J. Nanomed. 5 753
- [25] Manikandan R, Beulaja M, Prabhu N M, Thiagarajan R, Anjugam M, Palainsamy S, Sarvanan K and Arumugam M 2015 Spectrochim. Acta A 140 2
- [26] Prasannaraj G and Venkatachalam P 2017 Adv. Nat. Sci.: Nanosci. Nanotechnol. 8 025001
- [27] Vivek R, Thangam R, Muthuchelian K, Gunasekaran P, Kaveri K and Kannan S 2012 Proc. Biochem. 47 2405