

Anti-cancerous and Antioxidant activity of *Pergularia daemia* Inspired Zinc Oxide Nanoparticles against Lung Cancer (A549) Cell Line

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ABSTRACT

Background and Objectives: In nanotechnology, biosynthesis of nanoparticles has gained considerable attention. Development and synthesis of plant based metallic nanoparticles with improved properties and less undesirable effects have many advantages over the conventional methods. In addition to performing their intended function, these phyto-antioxidant functionalized nanoparticles can protect against oxidative damage. *Pergularia daemia* is a therapeutic plant that has been explored and identified with excellent pharmacological activity, but less reported. In this study, Zinc Oxide (ZnO) nanoparticles were manufactured from *Pergularia daemia* aqueous extract; the main objective was to evaluate their anticancer and antioxidant effects on the A549 cancer cell line. **Materials and Methods:** Aqueous leaf extract of *Pergularia daemia* was prepared, inspired with the nanoparticle and their properties were evaluated by FTIR and SEM. The cell viability and cytotoxicity of ZnO nanoparticles, at various doses, was confirmed by Trypan blue and MTT assay. The antioxidant property of ZnO nanoparticles was investigated by estimating the Reactive Oxygen Species, Lipid Peroxidase and Nitric Oxide estimation and BAX and PARP gene expression by Polymerase Chain Reaction (PCR). **Results:** A decreasing cell viability and increasing cytotoxicity was observed in green synthesized ZnO nanoparticles with increasing dose. Our data confirmed that ZnO nanoparticles enhances the antioxidant activity by scavenging the free radicals. Elevated apoptotic protein BAX with down regulated PARP was observed in green synthesized ZnO nanoparticles treated cells. **Conclusion:** Based on the above findings green synthesized ZnO nanoparticles with *Pergularia daemia* are effective against A549 cancer cell lines proving their anti-cancer and antioxidant activity.

Keywords: *Pergularia daemia*, Zinc Oxide Nanoparticles, A549 cell line, Anti-cancer activity, BAX gene, PARP gene.

Submission Date: 13-04-2022;

Revision Date: 17-05-2022;

Accepted Date: 03-06-2022.

INTRODUCTION

Nanotechnology represents a multidisciplinary field where nanostructures improve lives, from agriculture, water treatment, food industry, chemical and biological sensing, gas sensing, CO₂ capturing and drug delivery. Recently, nanoparticles have attracted more attention in biomedical field due to their noticeable characteristics and utilization.¹⁻² Among other capabilities, their drug delivering ability have increased their usage in medical field. Nanoparticles are categorised according to their properties, dimensions and sizes including polymeric, Carbon based, ceramic and metal nanoparticles.³

Owing to its biosafe and biocompatible properties ZnO nanoparticles are preferentially used in drug delivery. ZnO is inexpensive and exhibit unique properties such as better absorption of light, it has also been shown to have an *in vitro* impact on cancer cells.⁴ According to reports, ZnO nanoparticles generate reactive oxygen species (ROS),⁵ which can cause damage to the genetic material.⁶ To minimise the level of toxicity, a novel cost-effective, safe, and simple green approach has been developed, which is expected to be environmentally benign, non-toxic¹ with appreciable antimicrobial

DOI: 10.5530/ijper.56.3.138

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activity than chemically generated nanoparticles.⁷⁻⁹ Owing to their potential efficacy and zero side effects, medicinal plants were widely used and recently it has gained immense importance compared to modern synthetic drugs.¹⁰

Pergularia daemia (forsk, family Asclepiadaceae), a popular herbaceous climbing plant has high medicinal value and extensively used in traditional medicine for various human disorders. Among their various pharmacological functions, hepatoprotective, anticancer, antidiabetic, antioxidant, anti-inflammatory and cardiovascular effects have been reported in traditional medicine.¹¹⁻¹² A vast range of medically active chemicals, particularly glycosides, have been discovered by modern research. In fact, when ingested in excess, the plant is said to be poisonous, causing an instant and prolonged rise in carotid blood pressure (Protabase). The primary aim of the current study is to evaluate the antioxidant and anti-cancer capabilities of *Pergularia daemia* inspired Zinc Oxide Nanoparticles in lung cancer (A549) cell line.

MATERIALS AND METHODS

Sample Collection

The plant, *Pergularia daemia* was collected from Saudi online market. Extracts were prepared based on Naseer *et al.*¹³ without any modifications. The collected leaf samples were initially rinsed with tap water to eliminate surface contaminants; and distilled water before extraction. The samples were then air-dried (37°C) for a week. Five grams (5 g) of leaves were pulverized to fine powder, combined with 500 ml distilled water and heated at 70°C for 30 min. The extract was then filtered through a muslin cloth followed by filter paper (Whatman filter paper No.1). The extract was refrigerated (2-8°C) until further process.

Biosynthesis of Zinc Oxide nanoparticles

In a sterile 250 ml flask, ZnO nanoparticles were made by mixing 95 mL of 0.01M zinc acetate dihydrate solution with 5 mL of plant extract. This preparation was kept at 70°C for one hour with continuous agitation (150 rpm). A white precipitate of the biologically reduced zinc acetate dihydrate sedimented in the glassware. The effluent was discarded, and the precipitate was transferred to 1.5 ml microfuge tubes and centrifugated at 3000 rpm for 30 min with distilled water. To guarantee maximal impurity removal, this washing procedure was performed thrice.¹⁴

Characterization of ZnO nanoparticles

FTIR Analysis

The surface chemistry of the nanoparticles was investigated using FTIR analysis. In the range of 4000–500 nm, the surface of nanoparticles were observed for functional groups. The samples for this study were made by uniformly dispersing ZnO nanoparticles in a dry KBr matrix, subsequently crushed to produce a clear disc.

SEM analysis

About 1 mg of the biosynthesized nanoparticles was dispersed in 20 ml of methanol. On a carbon-coated grid, a thin layer of the material was prepared and dried under a mercury lamp. This preparation was taken for analysing the surface morphology of the nanoparticles under VEGA3TESCAN.

Cell culture and *In vitro* Cytotoxicity Assay

The cytotoxicity of the green synthesized ZnO nanoparticles was evaluated using A549 cells procured from ATCC (American Type Culture Collection – United States). These cells were grown in DMEM supplemented with 10% Fetal Bovine Serum and antibiotics (100 U/ mL Penicillin /100 µg/ mL streptomycin) and were incubated at 37°C with 5% CO₂. The effects of ZnO nanoparticles on A549 cell viability were investigated using trypan blue and the *in vitro* cytotoxicity assay by MTT method.

MTT Assay

The assay was performed based on Mosmann *et al.*, 1983,¹⁵ with slight variation. Briefly, A549 cells were harvested by trypsinization, counted and then diluted using DMEM. The cells were seeded (1×10⁴ cells/well) in 96-well plates and incubated for 24 hr. Following incubation, cells were microscopically analysed for attachment and treated with concentrations ranging from 10 to 100 µg/mL of the ZnO nanoparticles and incubated for 24 hr at 37°C. Next day, the cells were rinsed with 1x PBS, MTT (5 mg/ml) was added to each well, and the plates were incubated at 37°C for 4 hr, after which the formazan crystals were dissolved in 100 µl of DMSO. The resulting-coloured solution was analysed using a microplate reader at a wavelength setting of a wavelength of 490 nm and 630 nm. The control group consisted of untreated cells was considered as 100% of viable cells. Inhibition rate was calculated using the following formula:

$$\% \text{ Inhibition Rate} = \left\{ 1 - \frac{A_{490} - A_{630} (\text{Treated})}{A_{490} - A_{630} (\text{control})} \right\} \times 100\%$$

Cell viability assay

The toxicity of green synthesized ZnO nanoparticles on A549 lung cells was evaluated by trypan blue exclusion assay.¹⁶ Cells seeded in six-well plates, treated with varying concentrations of green synthesized ZnO nanoparticles and incubated for 24 hr (5% CO₂, 37°C). Following incubation, the cells were trypsinised, resuspended in equal volumes of culture medium and trypan blue solution. The relative proportion of live (unstained) and dead cells (blue) was estimated using a hemocytometer and calculated as given below.

Average number of cells per field = Sum of cells per field/Number of fields

Total number of cells per mL ($x * 10^4$ /mL) = Average number of cells per field \times dilution factor (2).

%Viability = Number of colourless cells/Total number of cells \times 100

Morphometric analysis by phase contrast microscopy

The morphological changes after treatment of A549 lung cancer cells with the various concentrations of *Pergularia daemia* inspired Zinc Oxide Nanoparticles was analysed using phase contrast microscopy. A549 cells from actively growing cultures were seeded into 6-well plate @ 1×10^4 cells/ml. The growth media was discarded after 24 hr and replaced with 100 μ l DMEM medium containing ZnO NPs (30, 40, and 50 g/ml) and incubated further for 24 hr. The morphological changes following this treatment was observed using a phase contrast microscope.

ROS (Reactive Oxygen Species) Assay

The Nitroblue tetrazolium (NBT) reduction assay was used to determine the effect of green synthesised ZnO-NPs on reactive oxygen species activity in A549 lung cancer cells. Free oxygen radicals convert NBT to create formazan, a blue-black chemical. This response is related to the cytoplasm's ability to generate reactive oxygen species (ROS).¹⁷ A549 cells from actively developing cultures were seeded at 1×10^5 cells/ml for this experiment. The growth media was discarded after 24 hr and replaced with 100 μ l DMEM medium containing ZnO NPs (30, 40, and 50 g/ml) and incubated further for 24 hr. After 24 hr, 0.1% of NBT solution was added after washing the cells with PBS (2X). After an hour, the cells were washed with 70% methanol (3X), followed by the addition of 2 M potassium hydroxide (120 μ l each) and DMSO. Absorbance was read at 630 nm.¹⁸

LPO (Lipid Peroxidase) Assay

Lipid peroxides degrade into a complicated succession of chemicals, including reactive carbonyl compounds, when exposed to air. The effect of the ZnO-NPs on the peroxidation of lipids in A549 cells was evaluated as in Okahwa *et al.*,¹⁹ with slight modifications. Briefly, A549 cells from actively growing cultures were seeded @ 1×10^5 cells/ml (duplicates). After 24 hr, the growth media was removed and 100 μ l DMEM medium containing ZnO nanoparticles (30, 40 and 50 μ g/ml) was added and further incubated for 24 hr. Following incubation, cells were washed with ice cold KCL buffer and centrifuged at 5000g for 5 min in ambient temperature. The cell pellet was re-dispersed with 0.2 ml of 8.1% SDS, 1.5 ml of 20% AA, 1.5 ml of TBARS solution and 0.7 ml of sterile water and incubated (95°C, 1 hr) and cooled to room temperature. The assay combination was centrifuged at 5000g for 20 min and quantified calorimetrically (OD = 532 nm). The standard was performed with MDA, treated and untreated samples, values expressed as μ M.

NO (Nitric Oxide) Assay

The impact of the green inspired ZnO nanoparticles on the nitric oxide scavenging potential of A549 lung cancer cells was estimated using the Griess-Ilosvay reaction.²⁰ This reaction quantifies the amount of nitrite ions produced when the nitric acid from sodium nitroprusside solution interacts with oxygen. For this assay, A549 cells from actively growing cultures were seeded @ 1×10^5 cells/ml. After 24 hr, 100 μ l DMEM medium containing ZnO nanoparticles (30, 40 and 50 μ g/ml) was added and treated for 24 hr. The spent media was used for assessing the NO-quenching potential of the nanoparticles. Equal amounts of medium and Griess reagent were combined and incubated at room temperature for 10 min in the dark. The reaction mixture is then tested at 540 nm for absorbance.

Gene expression analysis of BAX and PARP

A549 cells from actively growing cultures were seeded @ 1×10^5 cells/ml. After 24 hr, 100 μ l DMEM medium containing ZnO nanoparticles (30, 40 and 50 μ g/ml) was treated for 24 hr. DNA was isolated from cells, trypsinized from each treatment and control wells and processed for gene expression analysis using PCR.

The trypsinized cells were centrifugated (5 min, 1200 rpm), rinsed in phosphate-buffered saline (ice-cold) and re-centrifuged. After adding digestion buffer, the samples were incubated 16 hr in airtight tubes at 50°C. After incubation, the samples were centrifugated at 3000 rpm for 5 min at room temperature with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). The

resulting aqueous phase was combined with 7.5 M ammonium acetate and 100% ethanol, mixed and centrifugated (3000 rpm, 5 min) at room temperature. The pellet was cleaned in ethanol (70%) and dried in the open air. The air-dried pellet was dissolved in 30 μ L of nuclease-free water and Thermo cyclor gradient PCR was performed for β Actin (Forward-5' TCAAGGTGGGTGTCTTTCCTG3' and Reverse-5' TTTGCGGTGGACGATGGAG 3', BAX (Forward - 5' CGTGTCTGATCAATCCCCGA 3' and Reverse - 5' GAGGCCAGAAGGCAGGATTG 3') and PARP (Forward - 5' CCCAGCCTTGTGGAAAACAC 3' and 5' CACCTGCAGAGACAGGCATT 3') primers obtained through databases.

The reaction mixture consisted of Master mix (25 μ L), nuclease-free water (16 μ L), Sample template (5 μ L), Forward primer (2 μ L), Reverse primer (2 μ L) the with the total volume of 50 μ L. The initial denaturation step was set 95°C for 2 min. Annealing temperature was set 54°C for 30 sec for β Actin and PARP and 54.4°C for 30 sec for BAX. Extension cycle was set at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The PCR products were seen under UV light on a 1.0% agarose gel with ethidium bromide.

Statistical Analysis

GraphPad Prism was used to conduct the statistical analysis (Prism 5.1). One-way ANOVA was used to analyse the means of the collected data, followed by a Tukey multiple comparison test across groups. *P* value < 0.05 was considered as statistically significant. The results represent the mean values with standard deviation, obtained from three independent tests.

RESULTS

Characterization of Biosynthesized Zinc Oxide Nanoparticles

FTIR Analysis

Figure 1 represents the FTIR spectrum of biosynthesized Zinc oxide nanoparticles, showing the various functional groups. The C-Br stretch at 573.60 cm^{-1} is indicative of the presence of alkyl halides; the S-O stretch at 751.93 cm^{-1} and C-O stretch at 1000.01 cm^{-1} are indicative of sulfonates and anhydrides respectively. The C-N stretch (aryl) at 1335.57 represents amines.

The production of ZnO nanoparticles was suggested by spectral peaks between 691.58 and 570 cm^{-1} . The absence of peaks in the 3500 and 2500 cm^{-1} ranges indicated that aldehydes were not stretching in the typical OH and N-H ways. Methylene released by the proteins and C-N stretching patterns of amine are

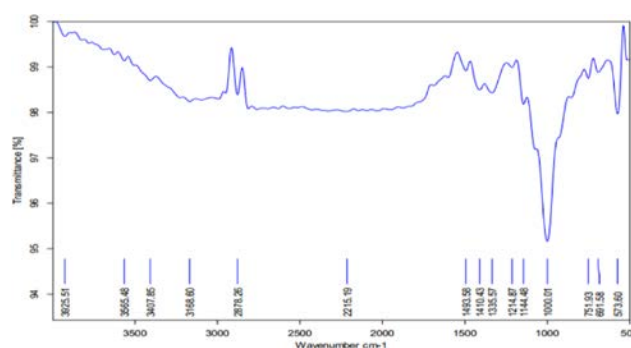


Figure 1: FTIR spectrum of *Pergularia daemia* inspired Zinc Oxide Nanoparticles.

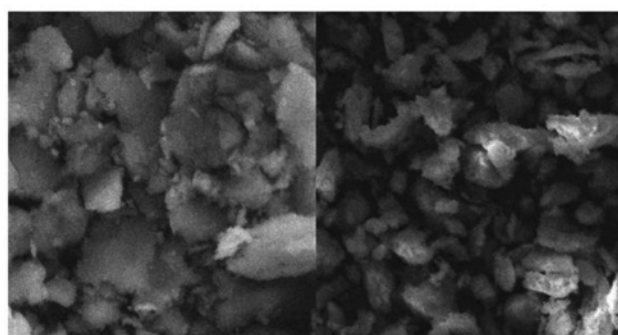


Figure 2: SEM images of *Pergularia daemia* inspired Zinc Oxide Nanoparticles (aggregated form).

represented by the bands from 1410 to 1000 cm^{-1} . Peaks at 1410-1335.57 cm^{-1} indicated the existence of C-H and O-H stretching vibrations in alkane and alcohol from leaf extract, stretching vibrations of amine. Peaks at 1410-1335.57 cm^{-1} suggested C-H and O-H stretching vibration of alkane and alcohol from leaf extract and bands at 1000.01 cm^{-1} demonstrated presence of sulfoxide and S=O stretching.

SEM Analysis

Figure 2 shows the morphology of biosynthesized ZnO nanoparticles, confirming the production of spherical and hexagonal nanoparticles grouped together with a rough surface.

In vitro Cytotoxicity Assay

MTT assay

The *in vitro* cytotoxicity of the *Pergularia daemia* inspired Zinc Oxide Nanoparticles was evaluated on A549 cells.¹⁵ The anticancer potential of the biosynthesised nanoparticles was determined using MTT assay. A dose-related reduction in viable cells was reported after a 24-hr treatment of A549 cells with varied concentrations of *Pergularia daemia* inspired Zinc Oxide nanoparticles (Figure 3). Based on these results, the IC_{50}

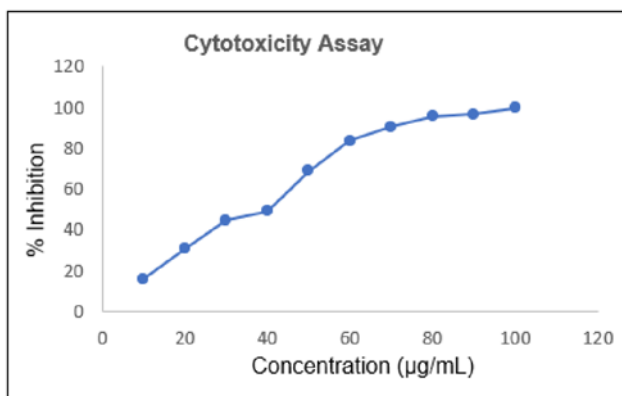


Figure 3: Cytotoxicity of *Pergularia daemia* inspired Zinc Oxide Nanoparticles on A549 Lung cancer cells – MTT assay.

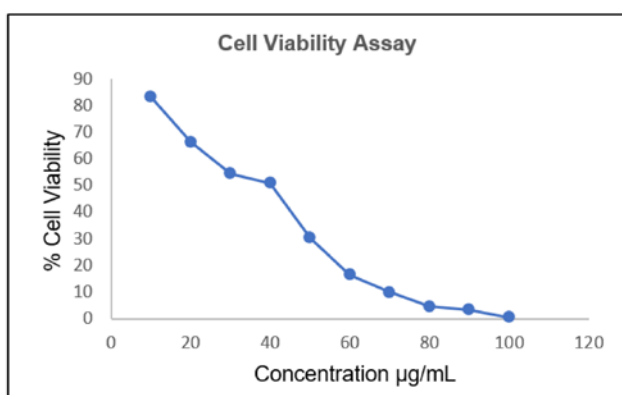


Figure 4: Effect of *Pergularia daemia* inspired Zinc Oxide Nanoparticles on A549 cells.

was determined. After 24 hr of exposure to the highest concentration of green synthesised ZnO nanoparticles (100 g/mL), 99.5% of A549 cells were dead. In trypan blue and MTT assay, about 50% of cell death was observed in 40 µg/ml of green synthesized ZnO nanoparticles.

Cell viability assay (Trypan blue assay)

The trypan blue dye exclusion method was used to assess the effect of *Pergularia daemia* inspired Zinc Oxide Nanoparticles on the cell viability of A549 human lung cancer cells. Concurring with the results of the *in vitro* cytotoxicity assay, the cell viability assay showed a dose-related decrease in the cellular viability after 24 hr exposure to green synthesized ZnO nanoparticles (Figure 4). About 83.1% of cells survived at the lowest dose of green produced ZnO nanoparticles (10 µg/ml). More than 95% of cells died at the highest concentration (100 µg/ml) of green synthesised ZnO nanoparticles, and A549 cells had a survival rate of 0.5%.

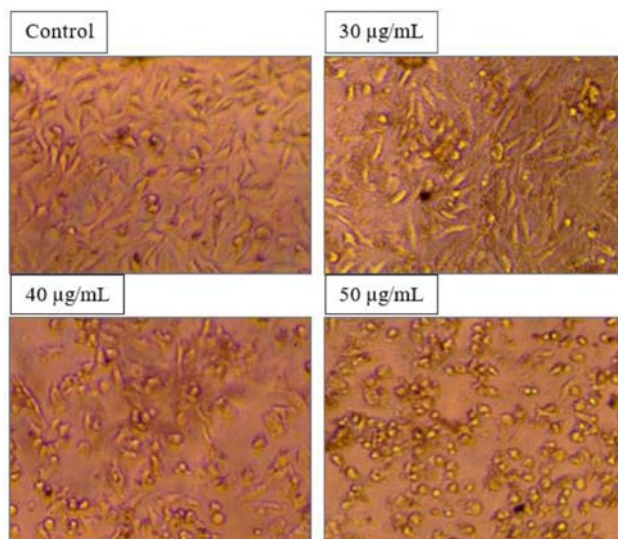


Figure 5: Phase contrast microscopic images (20 x) showing morphological changes of A549 cells upon treatment with *Pergularia daemia* inspired zinc oxide nanoparticles (30, 40 and 50 µg/ml for 24 hr).

Analysis of Morphological Changes by Phase Contrast Microscopy

Morphological changes were documented both in the untreated and cells treated with the nanoparticles (Figure 5). Untreated A549 cells appeared normal. Microscopic examination of cells revealed cell death in 50 µg/mL of green synthesized ZnO nanoparticles. The cells treated with the nanoparticles appeared shrunken, detached with their membranes blebbed and their morphology appeared deformed (Figure 5 – magnification -20 x; concentrations -30, 40 and 50 µg/mL for 24 h). With respect to the control, significant decrease was observed in cells treated with green synthesized ZnO nanoparticles. Phase contrast microscopy revealed intact cell morphology of control cells.

ROS (Reactive Oxygen Species) Assay

Reactive oxygen species (ROS) generation can result from incomplete oxygen reduction. Cells benefit from intracellular ROS levels that is below the functional limit. The anticancer and antioxidant activity of green synthesised ZnO nanoparticles in lung cancer cells was studied by reactive oxygen species (ROS). The green synthesised zinc oxide nanoparticles caused a dose-related increase in the accumulation of ROS in A549 lung cancer cells. The increase was statistically significant ($p < 0.0001$) at all the doses (Figure 6). In cells treated with nanoparticles, there was a nearly 2-fold rise in ROS levels, which was also statistically significant ($***p < 0.0001$).

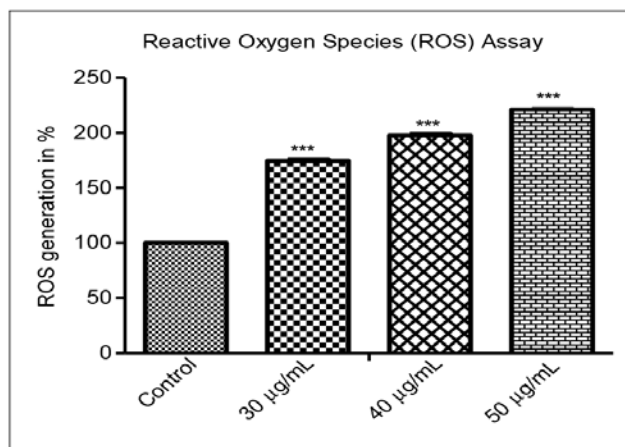


Figure 6: Effect of *Pergularia daemia* inspired Zinc Oxide Nanoparticles on the ROS production of A549 lung cancer cells.

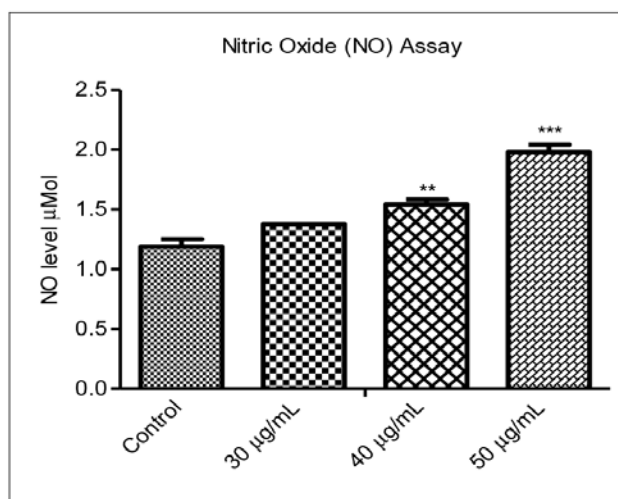


Figure 8: Effect of *Pergularia daemia* inspired Zinc Oxide Nanoparticles on nitric oxide production in A549 lung cancer cells.

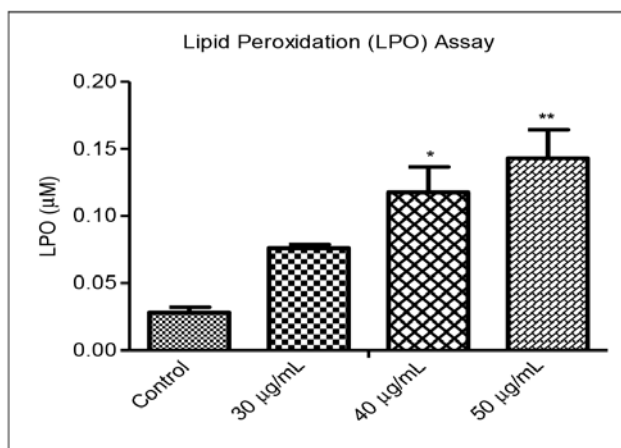


Figure 7: Effect of *Pergularia daemia* inspired Zinc Oxide Nanoparticles on lipid peroxidation of A549 lung cancer cells.

LPO (Lipid Peroxidase) Assay

The Effect of *Pergularia daemia* inspired Zinc Oxide Nanoparticles on lipid peroxidation of A549 lung cancer cells was estimated. The biosynthesised zinc oxide nanoparticles caused a dose-dependent increase in lipid peroxidation. The increase was statistically significant at 40 and 50 µg/mL (Figure 7). The lipid peroxidation exhibited positive correlation with ROS assay. In cells treated with green synthesized ZnO nanoparticles, there was a nearly 3-to-4-fold increase in lipid peroxidation, which was also statistically significant (***) with respect to the control.

NO (Nitric Oxide) Assay

Quenching of nitric oxide by *Pergularia daemia* inspired Zinc Oxide nanoparticles amplified with increase in dose (Figure 8). At 50 µg/mL, the quenching activity

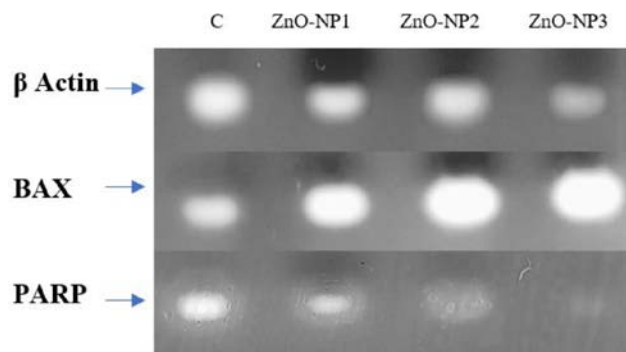


Figure 9: Analysis of BAX and PARP genes by PCR. With increasing dose, decreased gene expression levels for PARP were observed, while increased gene expression levels for BAX were documented. Housekeeping gene (β - Actin). Lanes C - Control, ZnO-NP1 - 30 µg/ml, ZnO-NP2 - 40 µg/ml and ZnO-NP3 - 50 µg/ml.

was substantially higher than the control, with a one-fold increase in NO level, which was also statistically significant (***) ($p < 0.0001$).

Analysis of BAX and PARP genes by PCR

The impact of nanoparticles on the molecular level was assessed using PCR-based gene analysis. The BAX and PARP, which are primarily involved in apoptotic gene signalling were analysed in A549 cells treated with *Pergularia daemia* inspired zinc oxide nanoparticles for 24 hrs. In the present study, BAX, the apoptotic gene was upregulated with increasing concentration and PARP, anti-apoptotic gene was downregulated with increasing concentration. This gene regulation pattern was observed to be in dose-dependent manner (Figure 9).

DISCUSSION

Recent developments in biosynthesis of nanoparticles have ecological and economic benefits over synthetic methods.³ Among several such nanoparticles, ZnO is considered the most promising materials in number of industries. *Pergularia daemia* is a therapeutic plant that has been explored and identified with excellent pharmacological activity, but less reported.²¹⁻²² Combining all these potentialities, the current study evaluated the antioxidant and anticancer potential of *Pergularia daemia* inspired zinc oxide nanoparticles on A549 cancer cell lines.

After confirming the formation of zinc oxide nanoparticles visually, the presence of various functional groups was identified using FTIR. The production of ZnO nanoparticles was suggested by spectral peaks between 691.58 and 570 cm^{-1} .²³ Amine, alkane, carbon dioxide, and alcohol,²⁴ are some of the major functional groups found in *P. daemia*, and they participate in the synthesis and stability of ZnONPs. All peaks in the FTIR spectra indicated a decrease in frequency, signifying, that the leaf proteins promote metal ion reduction,^{9,25} also aids in salt bio-reduction and nanoparticle capping.

Shape of the nanoparticle plays critical role against many factors. The morphological feature analysed through SEM confirmed that the nanoparticles synthesized with *P. daemia* are spherical in shape, clumped together, and rough on the surface.¹³ Studies have proven that spherical nanoparticles are more effective as they can easily penetrate the cell walls,²⁶ and that in malignant cells, the toxicity of ZnO nanoparticles is dependent on their size, with smaller nanoparticles being more toxic. Owing to this property, ZnO NPs synthesized with *P. daemia* can be of significance in treating cancer. The apoptotic induction of the green synthesized ZnO nanoparticles was evaluated in this research. In cells treated with higher concentrations of the nanoparticles, typical observations included rounding and shrinking of cells with apoptotic bodies, which is consistent with other verdicts in cancer cells.²⁷⁻²⁸

Cytotoxic evaluation of *Pergularia daemia* inspired zinc oxide nanoparticles revealed a concentration dependent cytotoxic response and has an IC_{50} of 50 $\mu\text{g}/\text{ml}$. In line with the existing reports,^{13,29} the survival of A549 cells decreased in a dose-related manner which is evidenced from the MTT assay. The trypan blue viability assay further confirmed this observation. The leaf extract of *Pergularia daemia* was tested for inhibiting ovarian carcinoma cell proliferation (PA-1 and OAW-42) in a study by Martin *et al.* with IC_{50} values of 30 and 120 $\mu\text{g}/\text{ml}$, respectively. The authors reported that

polyphenolic compounds present in the extract play an important part against tumour cell malignancy.³⁰ Furthermore, various authors have reported the anticancer potential of the plant. Methanolic extract of *Pergularia daemia* has been reported to have appreciable cytotoxic activity on human oral cancer KB cell line. An IC_{50} of 80 $\mu\text{g}/\text{mL}$ was reported in this stud. The extracts decreased the mitochondrial membrane potential.²⁹

In the current research, the antioxidant ability of green inspired ZnO nanoparticles was estimated and found to significantly alter A549 cells. ROS can activate the cascade of reduction-oxidation cycles within cells or across cell membranes, resulting in a depletion of cellular antioxidants and irreversible oxidative damage to cells. In comparison to untreated control cells, cells treated with 50 $\mu\text{g}/\text{ml}$ of green generated ZnO nano particles produced considerably more ROS and membrane lipid peroxidation. There was an increase in the amount of reactive oxygen species relative to the increase in concentration. Numerous reports have stated that ZnO nanoparticles cause the production of reactive oxygen species, which can lead to cell death if the cell's antioxidative capability is surpassed,³¹ the phenolic compounds in *P. daemia* may also be an attributable factor which can be highly correlated with antioxidant ability of the green synthesized ZnO. Furthermore, studies have revealed that *Pergularia daemia* at 80 $\mu\text{g}/\text{mL}$ caused 95% ROS generation in human oral cancer cells,²⁹ which is similar to our results. Increased production of intracellular ROS and lipid peroxidation in cellular membranes exposed to green synthesised ZnO nanoparticles suggest that oxidative stress is a major contributor to A549 cell damage.

Inflammation, cancer, and other pathological disorders have all been linked to nitric oxide, in addition to ROS. As highly reactive toxic gas which has free access to any subcellular compartment, nitric oxide has the ability to pass through any type of membrane system. Owing to its deleterious effects on the cellular process, we have evaluated the inhibitory effect of our green synthesized ZnO nanoparticles on A549 cells. With increasing sample concentration, a dose-related rise in NO production was found. This could be due to the fact that *Pergularia daemia* has the ability to reverse the effects of NO generation, which could be very useful in minimising the detrimental effects of excessive NO production in the human body. Furthermore, NO scavenging activity may help to halt chain reactions that are hazardous to human health and are initiated by excessive NO generation.

PARP (poly (ADP-ribose) polymerase) is a nuclear enzyme that helps fix DNA damages generated

by endogenous and external mechanisms.³²⁻³³ Moreover, PARP1 is directly involved in apoptosis and necrosis.³⁴ BAX, on the other hand is a protein involved in disrupting the membrane potential of the mitochondria and contributes to apoptosis. As *P. daemia* is said to damage DNA by initiating apoptosis in KB cell,²⁹ we evaluated the outcome of green inspired ZnO nanoparticle on A549 cells by quantifying the expression PARP and BAX. Our results indicated that the expression of PARP is decreased in a dose-related manner, whereas, the expression of BAX increased. As the nanoparticles suppress the expression of PARP, DNA repair would likely be adversely affected. NO, on the other hand, inhibits DNA mending, resulting in cell cycle arrest and apoptosis. According to studies, ROS causes phosphorylation of BAX, which causes cancer cells to die.³⁵ This is consistent with our findings, which show that as the concentrations of green inspired ZnO nanoparticles increased, the amounts of PCR products expressed from PARP and BAX decreased and increased, respectively. Polyphenolics (phenolic acid and flavonoids) found in *P. daemia* are expected to have played a key part in reversing the process by promoting apoptosis and preventing malignant cells from proliferating. According to the findings, *Pergularia daemia*-inspired zinc oxide nanoparticles cause cancer cell death in A549 cell lines by lowering cell proliferation, antioxidant status, increasing intracellular ROS and lipid peroxidation, and upregulating DNA damage and apoptosis.

CONCLUSION

Finally, for the first time, we successfully synthesised ZnO NPs from *Pergularia daemia* leaf extract and collected evidence of anti-cancer and antioxidant properties of the green produced ZnO nanoparticles in cancer cell lines. The green inspired ZnO nanoparticles produced cytotoxicity and inhibited cancer cell survival. Furthermore, the green synthesized ZnO nanoparticles increased ROS, LPO and NO levels in the cancer cells and caused apoptosis which was evidenced by PARP and BAX expression at the molecular level. To summarise, *Pergularia daemia* inspired ZnO nanoparticles may find significant use in cancer treatment.

ACKNOWLEDGEMENT

The author extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number RUP-5.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ZnO: Zinc Oxide; **FTIR:** Fourier Transform Infrared Spectroscopy; **KBr:** Potassium bromide; **SEM:** Scanning Electron Microscopy; **DMEM:** Dulbecco's Modified Eagle Medium; **MTT:** 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide; **PBS:** Phosphate Buffer Saline; **DMSO:** Dimethyl Sulfoxide; **ROS:** Reactive Oxygen Species; **NBT:** Nitroblue Tetrazolium; **ZnO-NPs:** Zinc Oxide Nanoparticles; **AA:** Acetic Acid; **SDS:** Sodium dodecyl Sulphate; **TBARS:** Thiobarbituric Acid Reactive Substances; **MDA:** Malondialdehyde; **KCL:** Potassium Chloride; **NO:** Nitric Oxide; **PCR:** Polymerase Chain Reaction; **ANOVA:** Analysis of Variance.

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PICTORIAL ABSTRACT



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SUMMARY

- Pergularia daemia* is a therapeutic plant with potent pharmacological properties. Zinc Oxide (ZnO) nanoparticles were successfully synthesized from the aqueous extract of *Pergularia daemia*. FTIR and SEM analysis confirmed the presence of nanoparticles. These biologically derived nanoparticles had a better cytotoxicity activity against lung cancer cells (A549). The ZnO nanoparticle elevated oxidative stress levels (LPO, NO, and ROS). Furthermore, apoptotic activity in A459 cancer cells was increased which was confirmed by the upregulation of BAX gene and downregulation of PARP gene. Overall, Green synthesized ZnO nanoparticles from *Pergularia daemia* have anti-cancerous effects.

Cite this article: Dailah HG. Anti-cancerous and Antioxidant activity of *Pergularia daemia* Inspired Zinc Oxide Nanoparticles against Lung Cancer (A549) Cell Line. Indian J of Pharmaceutical Education and Research. 2022;56(3):855-64.