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Original Article

Cytotoxic properties of *Pelargonium graveolens* leaf extract and its green-synthesized gold nanoparticles (in vitro study)

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الملخص

اهداف البحث: هدفت هذه الدراسة إلى تقييم التأثيرات السامة على الخلايا الحية لمستخلص من أوراق نبات العطرة وجسيمات الذهب النانوية الحيوية المصنعة بطريقة التوليف الاخضر لتكوين غسول الفم لاستخدامه كبديل لغسولات الفم التجارية. تم استخدام (الخلايا الليفية الجلدية البشرية من الاجنة) في هذه الدراسة التي أجريت في المختبر، لأن خصائصها كانت متطابقة تقريبًا مع خصائص الخلايا الليفية اللثوية البشرية.

طرق البحث: في هذه الدراسة، تم دراسة التوليف الأخضر لجسيمات الذهب النانوية الحيوية باستخدام مقتطفات من أوراق نبات العطرة كوسيلة مستدامة واقتصادية. بعد ذلك، وباستخدام مجموعة من التقنيات التحليلية، تم تقييم الخواص الفيزيائية والكيميائية، مثل المجهر الإلكتروني النافذ و حيود الأشعة السينية و ومطياف الامتصاص المرئي فوق البنفسجي، وبالتالي استغرقت الاستعدادات والتقنيات التحليلية لتكوين جسيمات الذهب النانوية وقتًا قصيرًا يبلغ حوالي عشرة أيام. وقد استخدمت الدراسة الحالية ٣-[٤,٥-ثنائي ميثيل ثيازول-٢-يل]-٢,٥-بروميد ثنائي فينيل-تترازوليوم (سمية التترازوليوم لموسمان) لدراسة التأثيرات السامة للخلايا لمستخلص أوراق نبات العطرة مع جسيمات الذهب النانوية الحيوية لنفس النبات باستخدام خط الخلايا القياسية المشتقة من الخلايا الليفية البشرية.

النتائج: تم استخدام جرعات مختلفة (١٠٠٠، ٥٠٠، ٢٥٠، ١٠٠، ٥٠، ٢٥، ١٢,٥، ٦,٢٥، و ٣,١٢٥ ميكروجرام/مل) من جسيمات الذهب النانوية لنبات العطرة لتقييم السمية الخلوية، وقد دلت النتائج على تأثيرات سامة خلوية قليلة (أقل من ٢٠٪ سمية تقريبًا). وقد لوحظ مستوى عالٍ من التوافق الحيوي بين جسيمات الذهب النانوية لنبات العطرة والخلايا الليفية البشرية الطبيعية.

الاستنتاجات: أظهر غسول الفم المصنوع باستخدام جزيئات الذهب النانوية الحيوية من مستخلص أوراق نبات العطرة المصنع بطريقة التوليف الاخضر, مستوى عالٍ من التوافق البيولوجي وله سمية خلوية منخفضة. لذلك، يمكن أن تكون تركيبات غسول الفم العشبية بمثابة بديل قابل للتطبيق لغسولات الفم الكيميائية.

الكلمات المفتاحية: نبات العطرة؛ جزيئات الذهب النانوية؛ غسول الفم؛ السمية الخلوية؛ لخلايا الليفية البشرية الطبيعية؛ سمية التترازوليوم لموسمان

Abstract

Objective: This study aimed to assess the cytotoxic effects of an extract from *Pelargonium graveolens* leaves and green-synthesized gold nanoparticles (AuNPs) in a mouthwash when used as a substitute for commercial mouthwashes. Human dermal fibroblasts from neonates (HDFn) were used in this study that done in vitro, because their characteristics were nearly identical to those of human gingival fibroblasts.

Method: In this study, the green synthesis of AuNPs using extracts from *P. graveolens* leaves was investigated as a sustainable and economical method. Then, using a range of analytical techniques, the physicochemical properties were evaluated, such as transmission electron microscopy (TEM), X-ray diffraction (XRD), and ultraviolet visible absorption spectroscopy (UV–Vis), so the preparations and analytical techniques of *P. graveolens* gold nanoparticles (AuNPs) take little time is about 10 days. The current study used the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl-tetrazolium bromide Mosmann's Tetrazolium Toxicity (MTT) to study the cytotoxic effects of *P. graveolens* leaf extract with *P. graveolens* gold nanoparticles (AuNPs) utilizing a human fibroblast-derived standard cell line.

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Results: Various doses (1000, 500, 250, 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL) of *P. graveolens* AuNPs were used to assess cytotoxicity, demonstrating little cytotoxic effects (approximately below 20% toxicity). A high level of biocompatibility was observed between the *P. graveolens* AuNPs and normal human fibroblasts.

Conclusion: The mouthwash made using green synthetic AuNPs obtained from *P. graveolens* leaf extract show high level of biocompatibility and has low cytotoxicity. Therefore, herbal mouthwash formulations can serve as a viable substitute for chemical mouthwashes.

Keywords: Cytotoxicity; Gold nanoparticle; Mosmann's tetrazolium toxicity; Mouthwash; Normal human fibroblast; *Pelargonium graveolens*

Introduction

Dental plaque (dental biofilm) is the primary etiological factor responsible for the development of periodontal diseases and tooth caries.[1] Periodontal disease is a widespread oral inflammatory disease often observed in middle-aged and elderly individuals. Prevention of periodontal disease may be achieved by early identification facilitated by advancements in clinical understanding and procedures.[2] Thus, it is essential to utilize efficient strategies for plaque management, for example, items like toothbrushes and dental floss to stop plaque formation and build-up on tooth surfaces. Mouthwashes can also be used as adjuncts to these mechanical methods, particularly in situations where people lack the necessary physical skills and drive, such as children. Chlorhexidine is generally recognized as the preferred mouthwash and is highly recommended as an effective antibacterial and antiseptic agent. However, the use of chlorhexidine has been associated with many undesirable consequences, including tooth discoloration, burning sensation, oral ulcers, and alteration in taste perception. These documented side effects require exploration of natural alternatives that are safe and minimize adverse reactions.[3]

Nanoparticles are widely used in biomedicine, medication administration, materials chemistry, and pollution prevention. Biosynthesized nanoparticles have been shown to have notable antibacterial and photocatalytic properties. Antibacterial activity, biosensing, optics, catalysis, and the treatment of diseases are just a few of the many uses for metal nanoparticles, such as iron oxide, zinc oxide, and gold nanoparticles.[4] In recent years, considerable interest has been demonstrated in the research of gold nanoparticles (AuNPs). AuNPs have unique properties, such as the ability to change size, large surface area relative to volume, remarkable optical properties, excellent compatibility with living organisms, low toxicity, and easy surface alteration.[5] Furthermore, nanoparticles have distinguishing properties, such as small size, large specific surface area, and the capacity to induce quantum phenomena.[6]

Due to their tiny size, nanomaterials possess a much higher surface area per unit mass in comparison to larger particles. As a consequence, there is a higher level of attraction between molecules and an exceptional capacity to evenly distribute in liquid mixtures. In addition, they have a significant affinity for proteins, plaque, and bacteria. Moreover, the enlarged surface area of the substance amplifies its antibacterial characteristics.[7]

Various methods have been used to fabricate nanomaterials, including chemical reduction, electrochemical synthesis, laser ablation, lithography, and pyrolysis. Generally, these methods are distinguished by their high expense and inherent hazards, mostly because of the use of hazardous reducing agents. The use of bio-fabrication by plants and other organisms to create nanoparticles offers a feasible, eco-friendly, economical, and sustainable method. Plant extracts provide prospective benefits in terms of the rapidity, ease, and appropriateness for nanoparticle production. Furthermore, plant phytoconstituents often serve as both lowering and stabilizing agents, resulting in decreased costs and improved effectiveness.[8] AuNPs have been synthesized from extracts of various wild plant species, including *Potamogeton pectinatus*,[9] *Ferula persica*,[10] *Phragmites australis*,[11] and *Pelargonium graveolens*.[12]

A variety of optical and physical methods are available for characterizing AuNPs. The distinctive optical and physical characteristics of AuNPs are mainly due to their peculiar shape, sizes, forms, and colloidal stability. Therefore, it is essential to characterize the produced AuNPs thoroughly utilizing a range of techniques in order to preserve their quality. In order to avoid unclear interpretations, a number of different approaches are usually advised for acceptable and complimentary characterizations.[13]

Nanoparticles material can be characterized by the following tests, Ultraviolet−Visible Spectroscopy (UV−VIS), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and X-ray Photoelectron Spectroscopy XPS.[14]

The plant species *P. graveolens* (Geraniaceae) is well-recognized for its production of a commercially popular essential oil, known as rose geranium. Its aerial components have been extensively used in traditional medicine. The oils and extracts *of P. graveolens* have been studied pharmacologically both in vitro and in vivo. The extracts' marker constituents include citronellol, guaia-6,9-diene, geranyl formate, linalool, isomethone, citronellyl formate, and geraniol.[15]

The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay is used for the in vitro evaluation of prospective pharmaceuticals and to investigate drug resistance in cellular models. Similarly, it aids in evaluating the effects of drugs in controlled laboratory environments and forecasting their actual use in clinical settings. This test is a method that is known for its high sensitivity, precision, and widespread usage in detecting cytotoxicity and assessing cell viability following exposure to harmful chemicals. Therefore, it is an invaluable tool for assessing the toxicity of substances and differentiating between various cell lines. It is efficient in

terms of cost, provides a large amount of data rapidly, and has exceptional sensitivity and reproducibility.[3]

Tests conducted in vitro can be used to estimate the toxicity of NPs in an initial manner. Realistically speaking, though, the intricate biological interaction seen in in vivo models does not correspond with the results obtained in laboratories. Consequently, a comprehensive biocompatibility testing program that includes an evaluation of the effects on organs and the immune system is necessary for the assessment of NMs. Any nanomaterial that might be used in the human body needs to be studied to find out if it can enter cells or tissues and how it gets dispersed, breaks down naturally, and gets eliminated. Therefore, it is indisputable that the use of animal models is significant and ought to be included in preclinical toxicity studies of nanoformulations.[16]

The current research seeks to examine the effects of AuNPs synthesized from *P. graveolens* leaf extract on a normal human cell line by assessing growth viability using the MTT assay. Therefore, this study has the potential to serve as a valuable resource for the development of sophisticated natural antibacterial treatments.

Materials and Methods

Materials and chemicals

The compound chloroauric acid ($HAuCl4 \cdot 3H_2O$) was acquired from Sigma-USA. Ethanol was obtained from Duksan (Seoul, Korea). Trypsin/EDTA (Ethylene diamine tetraacetic acid) reagent was procured from Capricorn, Germany, and DMSO (Dimethyl sulfoxide) was acquired from Santa Cruz Biotechnology (USA). The experiment utilized the minimum essential medium (MEM) obtained from Capricorn, Germany. The MTT stain used in the investigation was acquired from Bio-World-USA. Fetal bovine serum used in the experiment was sourced from Capricorn, Germany. The microtiter reader was sourced from Gennex Lab-USA, and cell culture plates were obtained from Santa Cruz Biotechnology, USA. All supplementary chemicals and reagents used in this study were of analytical grade about the characterization of the synthesized nanoparticles, using a Japanese X-ray diffract meter, Transmission electron microscopy TEM from PHILIPS in the USA is used to measure the size and shape of nanoparticles. A double-beam UV−Vis spectrophotometer was utilized to analyze beams ranging from 200 to 1100 nm for the experiment. The Zetasizer-Nano from ZS-Malvern Instruments was used to analyze the zeta potential. All materials and devices were summarized in Table 1.

Cell culture

For maintenance, a human dermal fibroblast from neonates (HDFn) cell line displaying typical features was grown in the minimum essential medium (MEM) enriched with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. The cells were propagated using Trypsin−EDTA and then replanted twice a week until they reached a confluence level of 50%. Afterwards, the cells were placed in an incubator set at a temperature of 37 °C.[17] The cell lines used in this study were obtained from the Cell Bank Unit of the Experimental Therapy Department at the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR). The cells are regularly assessed to determine their developmental features and undergo constant authentication.

Preparation of leaf extract from P. graveolens and the green synthesis of its AuNPs

The leaves of *P. graveolens* were collected from Diyala Governorate in Iraq. The leaves were carefully washed with sterilized water to remove any remaining chemicals and lime. Following a meticulous rinse, *P. graveolens* leaves left to dry overnight at a temperature of 37 °C. Following incubation, the thoroughly dried leaves were finely ground into a powder for extraction purposes. Then using 100 g of powder of plant material was extracted using aqueous alcohols as the solvent. The solvents used were methanol, water, and ethanol (1:1:3) 80% v/v, with a volume of 500 mL. The samples underwent Soxhlet extraction for 8 h in a water bath. Afterwards, the extracts underwent solvent removal by decreased pressure using a rotary evaporator at a temperature of 45 °C. Afterwards, the amounts of the dried, unrefined concentrated extracts were measured to calculate the yield. Subsequently, the powder was diluted to attain a concentration of 1 mg/mL, equivalent to the bactericidal concentration (MBC) of the plant extract. Subsequently, the filtered extracts were combined with 50 mL of gold chloride to serve as a reducing agent for the synthesis of gold nanoparticles. The gold ion solution was made following the approach described by Elia et al. (2014), with some alterations. A 10 mg/mL solution was prepared by dissolving (1 g of HAuCl4) in 100 mL of distilled water (DW). Gold nanoparticles (Au NPs) were synthesized by mixing a 1 mg/mL HAuCl4 gold ion solution with 50 mL of plant extract. The solution was vigorously stirred using a magnetic stirrer for 30 min while being heated slowly to a temperature ranging from 35 °C to 45 °C. Consequently, the mixture had a rapid alteration in color, shifting to a rich purple/red shade in a little duration. The color shift that was seen might be attributed to the production of 15.7 parts per million (ppm) of gold nanoparticles (AuNPs). The presence of a purple color, which arises from the amalgamation of the plant extract with the HAuCl4 solution, signifies the creation of *P. graveolens* AuNPs,[18] This substance is known for its high absorption in the ultraviolet (UV) region with a peak at 527 nm. The average size of nano crystallites in AuNPs is determined to be 69.587 nm using X-ray diffraction testing. TEM has shown the average size of AuNPs to be 19 nm, as seen in Figure 1. The zeta potential of AuNPs was determined to be 27 mV. This measurement indicates that the nanoparticles were stable and had a decreased tendency to clump together and form bigger particles. The preparing of *P. graveolens* AuNPs mouthwash is inexpensive, for example, the cost of preparing one liter was $30, so the economic effectiveness of this mouthwash within everyone's reach.

MTT assay

The cytotoxic assay is a screening test to evaluate the biocompatibility of the examined substances (*P. graveolens*

leaves extract and it,s AuNPs) in normal cell lines. In vitro evaluation of cytotoxicity can be performed by Mosmann's tetrazolium toxicity assay, which is considered the most common, rapid, and simple assay. This assay uses a yellow water-soluble dye, which converts to insoluble purple formazan.[19,20] The reaction occurs due to the fracturing of the tetrazolium ring by a mitochondrial enzyme called succinate dehydrogenase, which is exclusively present in healthy, viable cells.[21] This assay was conducted at the Biology Department of the College of Science, University of Diyala. For the cell suspension, 50 mL of tissue culture were mixed with a trypsin-versene solution. Afterwards, 20 mL of the culture medium, which was supplemented with the prepared serum, was added to the mixture. The cell suspension was mixed well and 0.2 mL was put into each well of the 96-well micro titer plates using a precise micropipette. Therefore, each well contained 1×10^5 (cells/well). The surface of the micro titer plate was covered with opaque transparent adhesive paper and the plate was moved gently for homogeneous distribution of cells in the wells. The plates had been Subjected to incubation at a temperature of 37 °C for a period of 12–18 h until the cells attached to the well. Consequently, the prior cultural medium was taken out from the wells. Then, 0.2 mL of the simultaneously formulated solutions of *P. graveolens* leaves crude alcoholic extract and *P. graveolens* AuNPs was added using serum free media (SFM) (6.25, 12.5, 25, 50, 100, 200, 250, 500,1000, 2000,4000,8000) μg/mL and deionized water as control negative, with three replicates for each concentration of each solution. After 72-h exposure time, after taking out the plate from the incubator, a precise volume of crystal violet dye, 50 μL, was carefully added to each well. The plate was left to incubate for 20 min. Afterwards, the contents were taken out, and the plate was rinsed with a phosphate buffered saline (PBS) solution as long as the excess dye was removed and the cells were allowed to dry. The crystal violet assay relies on the staining of cells that are adhered to cell culture plates. This process involves the separation of adherent cells from cell culture plates when cell death occurs. As part of the assay, non-viable cells are removed through a washing process, leaving behind only viable cells that are then stained using crystal violet. The crystal violet dye is solubilized, and its measurement is obtained by absorbance at 492 nm after the washing process. Cell biomass may be used to estimate cell viability along with cytotoxicity. The crystal violet test operates on the assumption that all cells adhered to the plate are viable, whereas any cells that detach are considered nonviable.

We used the following equation to calculate the cell growth inhibition rate (percentage of cytotoxicity):[22]

$$\textit{Cell viability}\ \% = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}}\ x\ 100$$

$$\text{Cytotoxicity}\ \% = 100 - \text{cell viability}$$

Statistical analysis

The significance level was determined using one-way analysis of variance (ANOVA) with a p-value of 0.05 to describe and analyze the data. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA, USA) was used to display the results as mean, standard deviation, and statistical significance.

Results

The formation of formazan crystals, which are produced when living cells reduce MTT tetrazolium, was used to measure cell viability. This research examined the impact of various doses of *P. graveolens* extract on the survival of normal human fibroblast cells by dose–response analysis. The objective was to calculate the IC50 value, which represents the concentration at which 50% of cell growth is inhibited. The results of this investigation are summarized in Table 1, where the data are shown as means accompanied by their corresponding standard deviations. The MTT test was employed to evaluate the cytotoxicity of various

Table 1: Materials and Equipments that used for cytotoxicity assay of *Pelargonium graveolens* leaf extract and its green-synthesized gold nanoparticles.

Equipment and materials	Company	Origin
Autoclave	Gallenkamp	United Kingdom
CO_2 Incubator	Gallenkamp	England
Crystal violate	BDH	England
Deep Freezer	The Electron Corporation	U.S. A
DMSO	Santacruz	USA
DPPH	Sigma	U.S. A
ELISA	Quik Fit	Germany
Fetal Bovine Serum	Sigma	U.S. A
Filter unit 0.22 μm	Millipore	Spain
Giemsa stain	Thomas Baker	India
Hot air oven	Memmert	Germany
Incubator	Memmert	Germany
Inverted Microscope	Olympus	Japan
Laminar Flow Hood	K & K	Korea
MEM	Capricorn	Germany
Micro Centrifuge	Hermle	Germany
Micropipette	Volac	England
Microtiter reader	Gennex Lab	USA
MTT Dye Sigma aldrich	Sluka	Germany
MTT stain	Bia-World	USA
HAuCl4	Sigma–Aldrich	U.S. A
pH-Meter	Radiometer	Denmark
Plastic Flasic For Tissue Culture	Falcon	U.S. A
RPMI-1640	Sigma	U.S. A
Sensitive Balance	Sartorius	Germany
Shaker Incubator	Selecta	Spain
Trypsin \ Versine	Sigma	U.S. A
Trypsin \ EDTA	Capricorn	Germany
Vortex	Griffin	England
Water Bath	Gallenkamp	England
An X-ray diffract meter (XRD-6000	SHEMADZU	Japan
Transmission electron microscopy	PHILIPS CM-120	U.S. A
UV–Vis spectrophotometer	SHEMADZU	Japan
Zetasizer Nano ZS	Malvern Instruments	Germany

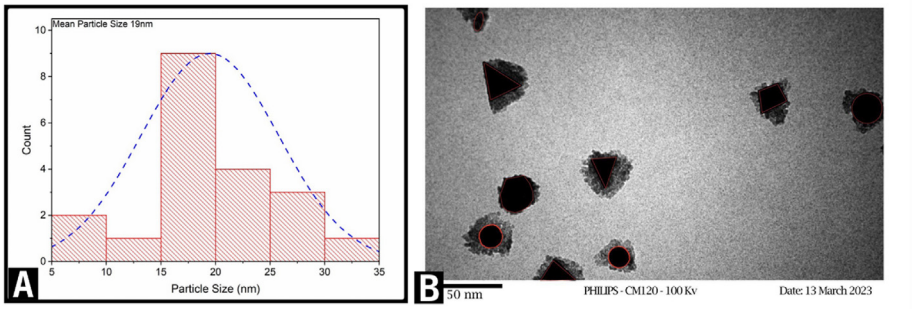

Figure 1: A-Distribution of particle size of *Pelargonium graveolens* AuNPs. B-TEM of *Pelargonium graveolens* AuNPs.

concentrations (8000, 4000, 2000, 1000, 500, 250, 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) of *P. graveolens* leaves extract on normal human fibroblast cells during a 72-h period. The concentrations of *P. graveolens* leaves extract at 250, 200, 100, 50, 25, 12.5, and 6.25 µg/mL exhibited reduced cytotoxicity towards the cells. The cytotoxicity of *P. graveolens* against typical human fibroblasts increased as its concentration increased. Furthermore, the test doses (50, 25, 12.5, and 6.25) µg/mL exhibited greater cell viability compared with that at 100, 200, 250, and 500 µg/mL concentrations, indicating a dose-dependent cytotoxic impact of the drug. As shown in Table 2, a noticeable increase in the

Table 2: Descriptive and statistical test of % of inhibition among agent and concentration (one way ANOVA).

Conc µg/mL	*Pelargonium graveolens* Extract Inhibition% ± SD	*Pelargonium graveolens* AuNPs Inhibition% ± SD	T test	p value
6.25	0 ± 0.0	0 ± 0.0		
12.5	0 ± 0.0	0 ± 0.0		
25	0 ± 0.0	0 ± 0.0		
50	0 ± 0.0	0 ± 0.0		
100	9.03 ± 1.1	0 ± 0.0		
200	10.30 ± 1.3	0 ± 0.0		
250	11.50 ± 1.5	0 ± 0.0		
500	16.35 ± 1.6	7.03 ± 1.1	8.313	**0.001HS**
1000	22.82 ± 1.8	9.03 ± 1.8	9.383	**0.0007HS**
2000	30.71 ± 1.9	15.71 ± 1.9	9.669	**0.0006HS**
4000	39.15 ± 2.0	20.15 ± 2.0	11.635	**0.0003HS**
8000	67.86 ± 2.3	40.86 ± 2.3	14.377	**0.0001HS**
F	399.678	158.149		
p value	**0.000**	**0.000**		

Sig. = significant at $p < 0.05$.

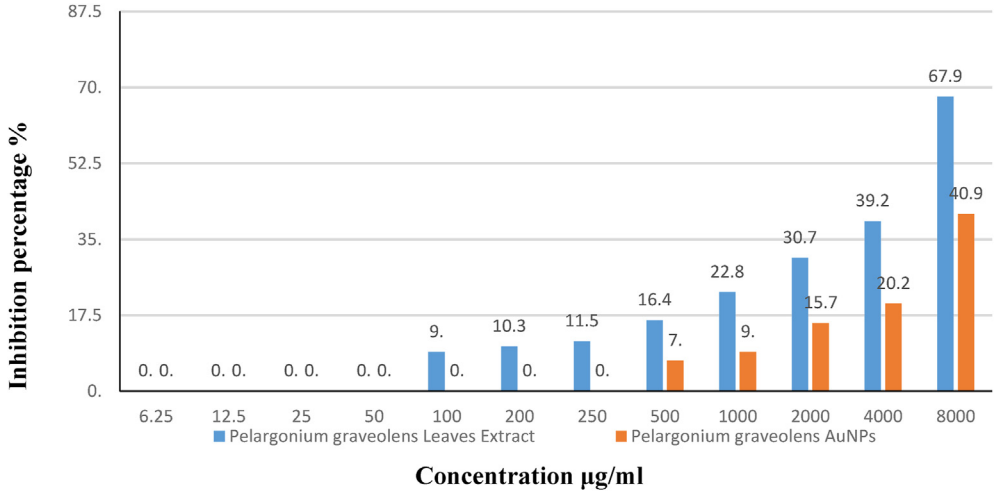

Figure 2: Mean of fibroblast inhibition percentage at different *Pelargonium graveolens* Leaves Extract and *Pelargonium graveolens* AuNPs concentration.

Table 3: Multiple comparisons of % inhibition between groups in *Pelargonium graveolens* Extract using Tukey HSD method.

Groups µg/mL	100		200		250		500		1000		2000		4000		8000	
	MD	p	MD	p	MD	p	MD	p	MD	p	MD	p	MD	p	MD	p
100			−1.27	0.98	−2.47	0.65	−7.32	**0.002HS**	−13.79	0.000HS	−21.68	**0.000HS**	−30.12	**0.000HS**	−58.65	**0.000HS**
200					−1.2	0.98	−6.05	**0.01HS**	−12.5	0.000HS	−20.41	**0.000HS**	−28.85	**0.000HS**	−57.38	**0.000HS**
250							−4.85	0.052	−11.32	0.000HS	−19.21	**0.000HS**	−27.65	**0.007HS**	−56.18	**0.000HS**
500									6.47	0.006HS	14.36	**0.000HS**	22.80	**0.000HS**	−51.33	**0.000HS**
1000											−7.89	**0.000HS**	−16.33	**0.000HS**	−44.86	**0.000HS**
2000													−8.44	**0.000HS**	−36.97	**0.000HS**
4000															−28.53	**0.000HS**

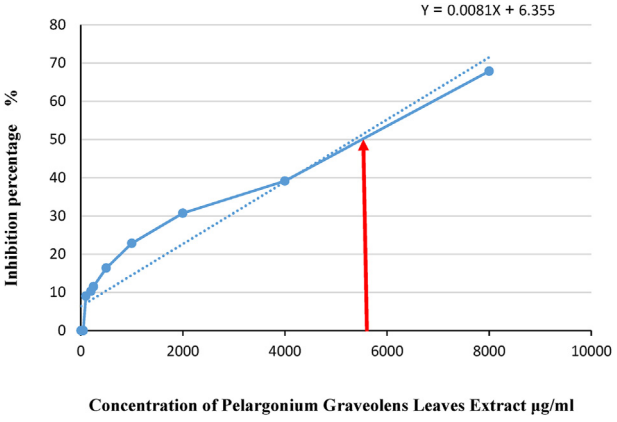

Figure 3: LC50 of *Pelargonium graveolens* Leaves Extract determination by simple linear regression graph.

mean fibroblast inhibition percentage was observed as the concentration of *P. graveolens* leaf extract increased. This increase was statistically significant, indicating that the cytotoxic impact of the extract depended on its concentration, as seen in Figure 2. Tukey's multiple pairwise comparison showed that the difference between each group with the other was significant except for between 100 vs. 200, 200 vs. 250, and 250 vs. 500 µg/mL (Table 3). In relation to the cytocompatibility of *P. graveolens* leaf extract, the determination of the half lethal concentration (LC50) required to cause the death of 50% fibroblast cells was achieved through the utilization of a simple linear regression equation (Y = 0.0081x + 6.355). This calculation was performed under the assumption that the value of Y corresponded to 50 (Figure 2). LC50 was determined to be 5388 µg/mL (Figure 3).

Table 4: Multiple comparisons of % inhibition between groups in *Pelargonium graveolens* AuNPs using Tukey HSD method.

Groups µg/mL	500		1000		2000		4000		8000	
	MD	p	MD	p	MD	p	MD	p	MD	p
500			−2	0.689	−8.68	**0.001HS**	−13.12	**0.000HS**	−33.83	**0.000HS**
1000					−6.68	**0.009HS**	−11.12	**0.000HS**	−31.83	**0.000HS**
2000							−4.44	0.089HS	−25.15	**0.000HS**
4000									−20.71	**0.000HS**

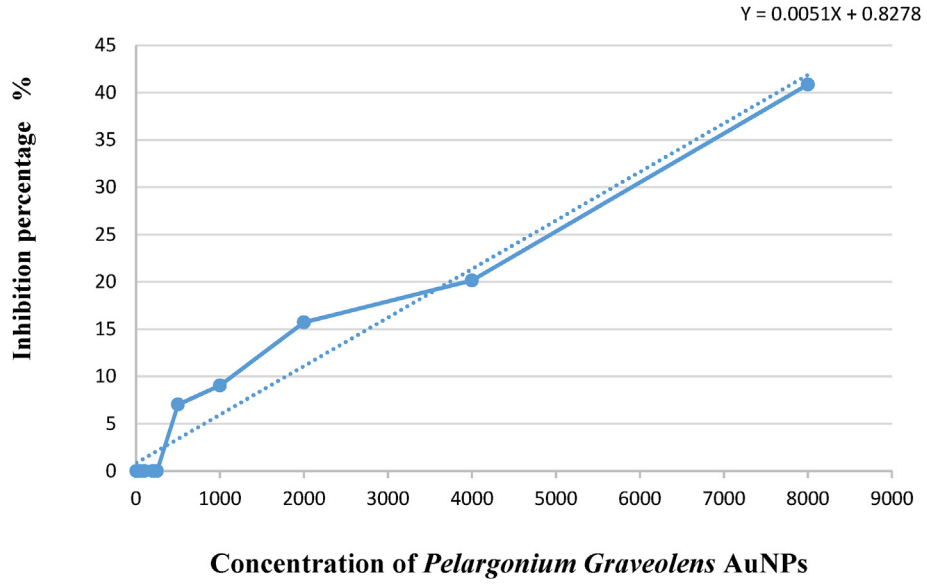

Figure 4: LC50 of *Pelargonium graveolens* AuNPs determination by simple linear regression graph.

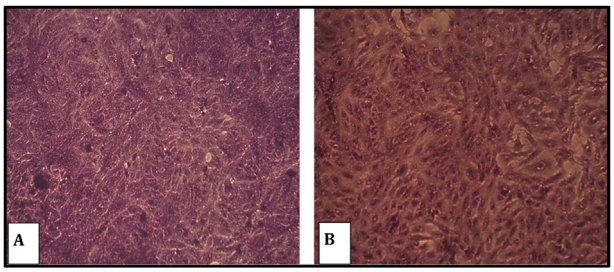

Figure 5: Normal morphology of human dermal fibroblasts cells (A) Untreated cells, (B) treated cells.

The MTT test was employed to explore the cytotoxicity of various concentrations (8000, 4000, 2000, 1000, 500, 250, 200, 100, 50, 25, 12.5, 6.25, and 3.125(µg/mL of *P. graveolens* AuNPs on normal human fibroblast cells in a 72-h period. The test doses 1000, 500, 250, 200, 100, 50, 25, 12.5, and 6.25 µg/mL exhibited greater cell viability compared with that at 8000, 4000, and 2000 µg/mL, indicating a dose-dependent cytotoxic impact of the drug. As shown in Table 2, there was a noticeable increase in the mean fibroblast inhibition percentage as the concentration of the *P. graveolens* leaf extract increased. Tukey's multiple pairwise comparison results showed significant differences between groups except for between 500 vs. 1000 and 2000 vs. 4000 µg/mL (Table 4). Regarding the cytocompatibility of *P. graveolens* AuNPs mouthwash, the LC50 was a very high concentration and all tested mouthwash concentrations killed less than 50% of fibroblasts, for example, a higher concentration of 8000 µg/mL killed 40% of fibroblasts as shown in Figure 4 the determination of the half lethal concentration (LC50) required to cause the death of 50% fibroblast cells was achieved through the utilization of a simple linear regression equation (Y = 0.0051x + 0.8278). This calculation was performed under the assumption that the value of Y corresponded to 50 (Figure 2). LC50 was determined to be 9641 µg/mL of *P. graveolens* AuNPs as shown in (Figure 4). And the cells visualized under phase contrast inverted microscope for normal morphology of human dermal fibroblasts cells as shown in Figure 5.

Also, the determination of the half lethal concentration (LC50) can be done using the most appropriate method) The probit analysis (is conducted by calculating the percentage of dead cells and then converting this percentage to a probit value using a recommended table. Then, will employ log values for the concentrations that were used to create a scatter diagram for the probability percent relationship. Subsequently, the equation (the Y values) is displayed, and the Y value is replaced with 5 (corresponding to a 50-lethal dosage). The X value (the log concentration) is then calculated using the equation (y = ax + b), and the log value is then converted to the absolute concentration, wherefore, Table 5 show that LC50 of *P. graveolens* leaves extract was about 2818.38 µg/mL, while, Table 6 show that LC50 of *P. graveolens* AuNPs was about 14791.08 µg/mL.

Table 5: The probit analysis to calculating the LC50 for different concentrations *Pelargonium graveolens* leaves extract.

Concentrations *Pelargonium graveolens* extract µg/mL	log 10 (concentration)	% dead	Probit
6.25	0.795880017	0	0
12.5	1.096910013	0	0
25	1.397940009	0	0
50	1.698970004	0	0
100	2	9.03	3.66
200	2.301029996	10.3	3.78
250	2.397940009	11.5	3.77
500	2.698970004	16.35	4.01
1000	3	22.82	4.23
2000	3.301029996	30.71	4.48
4000	3.602059991	39.15	4.72
8000	3.903089987	67.86	5.44
Intercept	−1.805683154		
X Variable 1	1.977674462		
y = ax + b			
y = 1.97x + (−1.80)			
5 = 1.97x − 1.80			
5 + 1.80 = 1.97x			
x=(5 + 1.80)/1.97			
x = 3.45			
LC50 = antilog x			
LC50 = antilog 3.45			
LC50 = 2818.38 µg/mL			

Table 6: The probit analysis to calculating the LC50 for different concentrations *Pelargonium graveolens* AuNPs.

Concentrations *Pelargonium graveolens* AuNPs µg/mL	Log 10 (concentration)	% dead	Probit
6.25	0.795880017	0	0
12.5	1.096910013	0	0
25	1.397940009	0	0
50	1.698970004	0	0
100	2	0	0
200	2.301029996	0	0
250	2.397940009	0	0
500	2.698970004	7.03	3.52
1000	3	9.03	3.66
2000	3.301029996	15.71	3.96
4000	3.602059991	20.15	4.16
8000	3.903089987	40.86	4.75
Intercept	−2.644881412		
X Variable 1	1.836876908		
Y = ax + b			
y = 1.83x + (−2.64)			
5 = 1.83x − 2.64			
5 + 2.64 = 1.83x			
x=(5 + 2.64)/1.83			
x = 4.17			
LC50 = antilog x			
LC50 = antilog 4.17			
LC50 = 14791.08 µg/mL			

Discussion

The MTT assay is widely used as a means of determining the cytotoxic impact of different chemicals over a range of circumstances or concentrations. This study used Human dermal fibroblasts from neonates (HDFn) because their characteristics are comparable to those of human gingival fibroblasts.[23] The IC50 value (half inhibitory concentration) of the applicable drugs can be calculated by examining the survival rates of the drug-treated and control groups.[19]

Therefore, we conducted laboratory tests to evaluate the cytotoxic effects of *P. graveolens* leaf extract AuNPs at different concentrations. The results of the experiments conducted on normal human dermal fibroblast cells over a 72-h period showed that the outcome was influenced by the dose of leaf extract administered. Small amounts of both tested solutions had minimal effects on cell viability. In contrast, when human cells were exposed to the IC50 dose, a significant decrease in cell viability was noted.

These findings suggest a direct relationship between the concentration of *P. graveolens* leaf extract and its level of toxicity. Concentrations of the herbal substance over 5330 μg/mL were specifically identified as more toxic. The results align with a previous study that investigated the cytotoxicity of *P. graveolens* leaf extracts using in vitro tests.[24] Furthermore, the use of *P. graveolens* extract has gained extensive recognition in the food industry owing to its established safety and approval by the American Food and Drug Administration (FDA) for use in food products.[25] The use of botanical medicines for the management and prevention of oral ailments might provide sporadic benefits to rural communities or individuals with limited financial resources, owing to the cost-effectiveness and widespread accessibility of herbal resources throughout the nation. Consequently, medicinal plants have the potential to serve as feasible alternatives to conventional antibacterial agents.[26] In addition, some adverse effects are linked to the chemical substances included in some drugs currently used, and should be avoided.

Several studies have investigated the use of *P. graveolens* extract for the green syntheses of AuNPs and have demonstrated its antibacterial properties. Therefore, this research seeks to investigate the possible harmful effects of these medications. This investigation is relevant for determining its appropriateness for patient use and for establishing its efficacy as a mouthwash at a specified concentration, which is considered the standard.

The main aim of the research was to produce AuNPs to examine their potential use in biological systems. *P. graveolens* AuNPs were thoroughly tested for biocompatibility and cytotoxicity. Different dosages (100, 50, 25, 12.5, 6.25, and 3.125 μg/mL) of *P. graveolens* AuNPs were employed to measure cytotoxicity and demonstrated slight cytotoxicity (approximately 20% toxicity). The findings presented in this study indicate a high level of biocompatibility between the AuNPs and normal human fibroblasts. The results align with the findings presented by Elia et al., in 2014.[18]

Furthermore, over the last few years, several pharmaceutical firms have obtained permission from FDA for the development of nanotechnology-based pharmaceuticals. This is primarily driven by the pressing need for substantial investment in the creation of novel nanotechnology-based medical devices for therapeutic purposes.[27]

Study limitation

1. Due to lack of facilities and abilities make it difficult to expose any hazardous medications that could potentially induce a condition resembling periodontitis, which will make the experiment's applicability more realistic by testing the cytoprotective effect of prepared nano-based mouthwash after infecting cells with a harmful medication, as this will simulate the patient's condition. Thus, it could be adapted for clinical applications. This will need more studies in future.
2. Unavailable kits and materials at Iraqi laboratories; Therefore, it needs to be imported from outside Iraq, and this takes a lot of time, outside the specified study time.
3. Conducting research with many repetitions the cytotoxicity test can be expensive and resource-intensive and this research has limited funds.
4. Unavailable the following tests in Iraq (Flow cytometry−fluorescent assays−Luminescent assays−Dye exclusion assay−Dye uptake and release assay) employing any of these techniques in future studies will improve the validity and reliability of cytotoxicity measurements. Other studies will be conducted if these capabilities are available in our country. Note that it is a novel study that is being conducted for the first time.

Conclusion and recommendations

In the current study, examination of the alcoholic crude extract of *P. graveolens* and it's green synthetic AuNPs revealed varying levels of cell viability based on the doses used. Additionally, we assessed the effect of these substances on cell viability, and found that a normal number of human fibroblasts remained stable with a very small decrease in response to low doses., indicating a dose-dependent hazardous impact. *P. graveolens* AuNPs demonstrated minimal cytotoxicity in human fibroblast cells, allowing their use in dentistry based on recommendations due to their potential as a viable substitute for chlorhexidine gluconate 0.12% (a common oral antibacterial mouthwash) for both the prevention and treatment of bacteria-induced diseases.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The ethical committee of the University of Baghdad's-College of Dentistry, gave consent for the study (No. 771233, 26-1-2023 with Ref. number: 771).

Authors contributions

The research report was written by AYM and AHMJ, who also helped with the study's design, implementation, and interpretation of data. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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