Hindawi
Journal of Nanotechnology
Volume 2018, Article ID 9689131, 11 pages
https://doi.org/10.1155/2018/9689131

*Research Article*

# Biological Effect of Organically Coated *Grias neuberthii* and *Persea americana* Silver Nanoparticles on HeLa and MCF-7 Cancer Cell Lines

**Lizeth Salazar,[1] María José Vallejo López,[2] Marcelo Grijalva,[1,2] Luis Castillo,[3] and Alexander Maldonado[1]**

[1]*Centro de Nanociencia y Nanotecnología, Universidad de las Fuerzas Armadas ESPE, Avenida General Rumiñahui S/N y Ambato, P.O. Box 171-5-231B, Sangolquí, Ecuador*
[2]*Departamento de Ciencias de la Vida, Universidad de las Fuerzas Armadas ESPE, Avenida General Rumiñahui S/N y Ambato, P.O. Box 171-5-231B, Sangolquí, Ecuador*
[3]*Facultad de Ingeniería, Ciencias Físicas y Matemáticas, Universidad Central del Ecuador, P.O. Box 1701521, Quito, Ecuador*

Correspondence should be addressed to Marcelo Grijalva; rmgrijalva@espe.edu.ec

Received 8 March 2018; Revised 5 June 2018; Accepted 2 July 2018; Published 1 August 2018

Academic Editor: Lee Blaney



The aim of this study was to assess the biological effect of organically coated *Grias neuberthii (piton)* fruit and *Persea americana (avocado)* leaves nanoparticles (NPs) on cervical cancer (HeLa) and breast adenocarcinoma (MCF-7) cells with an emphasis on gene expression (p53 transcription factor and glutathione-S-transferase *GST*) and cell viability. UV-Vis spectroscopy analysis showed that synthesized AgNPs remained partially stable under cell culture conditions. HeLa cells remained viable when exposed to piton and avocado AgNPs. A statistically significant, dose-dependent cytotoxic response to both AgNPs was found on the breast cancer (MCF-7) cell line at concentrations above 50 $\mu$M. While expression levels of transcription factor p53 showed down-regulation in treated MCF-7 and HeLa cells, *GST* expression was not affected in both cell lines treated. Cell viability assays along with gene expression levels in treated MCF-7 cells support a cancer cell population undergoing cell cycle arrest. The selective toxicity of biosynthesized piton/avocado AgNPs on MCF-7 cells might be of value for novel therapeutics.

## 1. Introduction

Breast and cervical cancers are the most common malignancies among females in low- and middle-income countries (LMICs). The two malignancies are associated with high mortality rates and represent a considerable burden for public health systems [1]. Currently available cancer therapeutics, such as chemotherapy and radiotherapy, exhibit limitations that must be overcome to improve their efficacy and patient's life expectancy. Since cancer is a world health problem, emerging drug preparations that can pass through tumor barriers and enhance anticancer drug delivery are potentially useful [2]. In this context, a lot of research has been done to synthesize new classes of materials, including those at nanoscale, and test their anticancer properties and/or their application in cancer early detection approaches [3, 4]. Silver nanoparticles (AgNPs) are widely applied in cancer research due to their potent *in vitro* antitumor effects on cancer cell lines including breast and cervical cancer models [5, 6]. The cytotoxic response that a nanoparticle could trigger in cells depends not only on its physical and chemical characteristics [3], but also, since different cell lines do not respond identically to stimuli with the same nanoparticles, on the cell type [7].

The synthesis of AgNPs through different physical, chemical, and biological methods and with well-defined parameters of size and shape has been reported by several authors [8]. Recent studies suggest that biosynthetic

approaches for AgNPs fabrication may improve some limitations found with commonly used physical and chemical methods, namely, high-energy consumption, negative environmental impact, and significant production costs [3, 8]. Plant extracts are commonly used as reducing and stabilizing agents for the biosynthesis of AgNPs. The cytotoxic and antiproliferative effects of plant-based AgNPs against different cancer cell models have been extensively investigated [9]. A study using green synthesis with traditional plants is the one performed by Barua et al., where *Thuja occidentalis* leaf extract was used to synthesize AgNPs that displayed anticancer properties against MCF-7, MDA-MB-231, KB, and HeLa cell lines. NPs also showed antibacterial properties against *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium,* and *Pseudomonas aeruginosa* [29].

An interesting finding has been an upregulation of proapoptotic genes following AgNPs exposure [10–12]. A recent study found that AgNPs may induce cell death through the p53 apoptotic pathway in a time- and dose-dependent manner [13, 14]. The tumor suppressor p53 gene induces cell cycle arrest and triggers apoptosis initiation in cells with extensive DNA damage. In some types of cancer, however, p53 inactivation functions as a drug-resistance mechanism [15]. Thus, *in vitro* expression studies of p53 are important to evaluate AgNPs cytotoxicity.

Oxidative stress is another harmful effect of AgNPs. In response to high rates of reactive oxygen species (ROS), oxidative stress-related genes (catalase, mu class of glutathione-S-transferase) are reported to be overexpressed [16]. The last one, glutathione-S-transferase (*GST*), is an antioxidant defense enzyme that catalyzes the coupling of reduced glutathione to a variety of damaging compounds to activate cellular outflow of these contaminants [17].

*P. americana* (avocado) is a member of the *Lauraceae* family, which has been used in herbal medicine in Central and South America due to its pharmacological properties [18, 19]. It is well known that avocado could exert antioxidant, anti-inflammatory, and other beneficial effects [19]. The avocado pulp, seeds, and leaves contain lipophilic phytochemicals [20] and phenolic compounds [21]. Anitha and Sakthivel have reported the biosynthesis of AgNPs using aqueous leaf extract of avocado as reducing agent and demonstrated an anti-inflammatory effect on red blood cells [22]. Another study showed that biosynthesized AgNPs from avocado showed strong antimicrobial effect against gram-positive and gram-negative bacteria [23].

*G. neuberthii* (Sachamango, piton) is a medicinal tree, belonging to the *Lecythidaceae* family, that grows in the Amazon regions of Peru, Brazil, and Ecuador [24–26]. Traditional medicine in local indigenous communities uses piton properties for treatment of several pathological conditions including sinusitis, uterine bleeding, diarrhea, constipation, among others [27]. Recently, Vásquez-Ocmín and coworkers demonstrated that a *G. neuberthii* bark extract had antiparasitic activity *in vitro* [28]. The application, however, of *G. neuberthii* extract in the synthesis of AgNPs has not been reported yet.

The present study aimed at evaluating the effects of two types of biosynthesized AgNPs using *G. neuberthii* fruit and *P. americana* leaf extracts (as stabilizing and reducing agents) on breast (MCF-7) and cervical (HeLa) cancer cell lines. NPs' cytotoxicity was assessed using an MTT colorimetric assay. The study also assessed the AgNPs modulation properties in two metabolic pathways: apoptosis and oxidative stress. For this purpose, gene expression assays were used for relative quantification of the expression of p53 and *GST* genes.

## 2. Materials and Methods

*2.1. Silver Nanoparticles (AgNPs).* *G. neuberthii* fruit AgNPs and *P. americana* leaf AgNPs were biosynthesized and kindly provided by Dr. Brajesh Kumar from the Advanced Materials Laboratory at CENCINAT, Universidad de las Fuerzas Armadas ESPE, Ecuador. AgNPs were characterized by transmission electron microscopy (TEM) (FEI-TECNAI G20 SPIRIT TWIN, USA), UV-visible spectroscopy (Analytik Jena SPECORD® S 600, Germany), and dynamic light scattering (DLS) (HORIBA LB-550, Japan). *G. neuberthii* and *P. americana* AgNPs were spherical, and their hydrodynamic diameters were $38.9 \pm 19.0$ nm and $41.1 \pm 19.1$ nm, respectively.

*2.2. Cell Culture.* MCF-7 (human breast adenocarcinoma) and HeLa cell (cervix adenocarcinoma) lines were kindly provided by Dr. Javier Camacho, CINVESTAV-IPN, Mexico. The cell lines were cultivated in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F-12) (Gibco), supplemented with 10% fetal bovine serum (FBS) (Gibco), and 1% penicillin-streptomycin (Gibco). Additionally, 1% sodium pyruvate (Gibco) was added to HeLa cell medium. Cell cultures were maintained at 37°C and 5% $CO_2$ in a humidified atmosphere. Subcultures were obtained when cells reached 85–90% confluence. Cells were washed three consecutive times with phosphate-buffered saline (PBS) (Gibco) and then detached with trypsin-EDTA 0.25% solution (Gibco). Cellular suspensions were centrifuged at 1000 rpm at 22°C for 10 minutes. Pellets were resuspended in medium, and cells were counted on Neubauer chambers with an inverted microscope (MICROS Austria MCX-1600, Austria). Cells were seeded on 6-well plates or 60 mm × 15 mm Petri dishes at specific densities.

*2.3. Nanoparticles' Stability under Cell Culture Conditions.* Prior to *in vitro* biological testing, the stability of the two types of biosynthesized AgNPs (*G. neuberthii* and *P. americana*) under cell culture conditions was assessed by UV-visible spectroscopy (Analytik Jena SPECORD S 600, Germany). Stock AgNPs preparations (1 mM) were diluted in complete culture medium and distilled water. AgNPs solutions were placed in 60 mm × 15 mm Petri dishes and incubated at 37°C and 5% CO2 for 0, 24, and 48 hours. An additional control was included to evaluate the influence of cellular debris. For this control, MCF-7- HeLa cell lines were grown for 24 hours and then exposed to NPs. The supernatant was later collected

to determine the presence of agglomeration. For noncontrols, MCF-7 and HeLa cell lines were seeded ($1.5 \times 10^5$ cells) and maintained for 24 hours, followed by exposure to different AgNPs concentration and incubation times. Finally, UV-visible absorption spectra were measured at 350–800 nm.

*2.4. MTT Assay for Cytotoxicity Assessment of Nanoparticles.* MCF-7 and HeLa cells were seeded in 96-well plates in 200 $\mu$L of complete culture medium at a density of $4 \times 10^3$ cells per well and $2 \times 10^3$ cells per well, respectively. Cells were maintained under culture conditions as described above. Different concentrations of *G. neuberthii* and *P. americana* AgNPs were used. Dilutions of AgNPs were obtained adding complete culture medium to final exposure concentrations of 1–80 $\mu$M for MCF-7 cells and 0.001–50 $\mu$M for HeLa cells. For each cell line and AgNPs type, six technical replicates were performed. The complete experiment was carried out three times (biological replicates). Blank wells containing only medium and untreated cells were included in every experiment, as appropriate controls. After an exposure time of 48 hours, cell viability was measured by a 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT), colorimetric assay (Molecular Probes), following the manufacturer's recommendations.

For the MTT assay, supernatants were removed, and 100 $\mu$L of Dulbecco's Modified Eagle's Medium (DMEM) without red phenol was placed in each well. A 12 mM MTT stock solution was prepared by adding 1 mL of sterile PBS. Next, 10 $\mu$L of this stock was added to each well. Cells were then incubated protected from light for 4 hours, followed by addition of 100 $\mu$L of SDS-HCl and homogenization and another 4-hour incubation, in order to dissolve intracellular formazan products. Finally, absorbance was measured at 570 nm using a microplate reader (Perlong, Beijing). Cell viability was calculated as the ratio of the mean absorbance obtained for the treatment wells to the mean absorbance of the control wells (untreated cells) as follows:

$$\text{cell viability}\,(\%) = \frac{\text{mean absorbance of treatment}}{\text{mean absorbance of control}} \times 100\%. \quad (1)$$

*2.5. Quantitative Real-Time PCR Analysis.* MCF-7 and HeLa cells were seeded into 6-well plates at a cell density of $1.5 \times 10^5$ per well. After a 24-hour culture period, cells were exposed to different concentrations of AgNPs for 48 hours. Tested AgNPs concentrations were 0, 40, 80, and 160 $\mu$M for the MCF-7 cell line, and 0, 25, 50, and 100 $\mu$M for the HeLa cell line. After 48 hours of exposure to either piton or avocado AgNPs, cells were collected and diluted in 200 $\mu$L PBS. Total RNA was then extracted using *PureLink® RNA Mini Kit* (Ambion) and purified with *TURBO DNA-free™ Kit* (Ambion) in order to remove contaminant genomic DNA. Purified RNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Scientific) and also separated by electrophoresis in an agarose gel (1%) with 1X Tris-borate-EDTA buffer (TBE) at 100 V and 300 mA for 55 minutes to check for integrity.

Purified RNAs were used in one-step qRT-PCR assays on a LightCycler® Nano Instrument (Roche Diagnostics), with TaqMan probes chemistry to determine relative quantification of p53 and *GST* genes in MCF-7 and HeLa cell lines. Primers and probes used in the present study are listed in Table 1. TaqMan probes (Invitrogen) were end-labeled with the fluorophore 6-carboxyfluorescein (6-FAM) and the quencher tetramethylrhodamine (TAMRA) at 5' and 3', respectively. Each PCR mix included 1X TaqMan® RT-PCR Mix, 0.5 $\mu$M of forward primer, 0.5 $\mu$M of reverse primer, 0.15 $\mu$M of TaqMan probe, 1X TaqMan RT Enzyme Mix, DEPC-treated water (Invitrogen), and 20 ng RNA template in a total volume of 10 $\mu$l. The qRT-PCR thermocyling program was as follows: cDNA synthesis at 48°C for 15 min, followed by a polymerase activation step at 95°C for 10 minutes. Then, 40 cycles of thermal amplification were carried out at 95°C for 15 s (denaturation), 50°C or 56°C for 15 s (for primer annealing of *ACTB* and *GST* genes, resp.), and a last step of 45 s at 60°C (extension). For p53 gene amplification, annealing and extension were performed in a single step at 61°C for 60 s. For PCR efficiency and correlation coefficient ($R^2$) calculations, 10-fold RNA dilutions were used. A semilogarithmic graph was constructed with Ct values plotted on the *X*-axis and log values of the RNA concentration, on the *Y*-axis. qRT-PCR efficiencies were calculated according to the following equation:

$$E = 10^{[-1/\text{slope}]}. \quad (2)$$

p53 and *GST* expression levels were normalized with actin beta gene (housekeeping gene) expression levels according to the methodology developed by Pfaffl (2001) [30]. RNA expression levels from untreated cells were used as calibrators to calculate the relative expression ratios.

*2.6. Statistical Analysis.* Six replicates of each treatment were done for cytotoxicity experiments. For gene expression qRT-PCR, experiments were performed in triplicate. Data are presented as the mean ± standard deviation (SD). The Kruskal–Wallis, Dunn's, and Tukey's multiple comparison tests were performed to determine any significant difference between controls and treatments. A $p$ value <0.05 was set for statistical significance.

## 3. Results

*3.1. AgNPs Stability.* Stability of biosynthesized *G. neuberthii* AgNPs and *P. americana* AgNPs in biological medium and distilled water was evaluated by UV-visible spectroscopy for 3 incubation times under standard conditions of 37°C and 5% $CO_2$ in a humidified atmosphere (see supplementary data (available here)). Results showed that absorbance values were higher when AgNPs were diluted in culture medium in comparison with AgNPs diluted in distilled water. UV-Vis spectra of all samples showed a broad peak at the maximum absorption wavelength between 400 and 420 nm due to the surface plasmon resonance (SPR) band of spherical AgNPs.

Table 1: Oligonucleotide sequences for quantitative, real-time PCR analysis.

| Gene name | Sequences (5′-3′) | Tm (°C) | GenBank (accession code) | Amplicon size (bp) | Efficiency | Reference |
|---|---|---|---|---|---|---|
| Glutathione-S-transferase (*GST*) | Forward: GATACTGGGGTACTGGGACATCC | 61.58 | NM_146421.2 | 130 | 1.87 | [31] |
| | Reverse: CCACTGGCTTCTGTCATAATCAGG | 61.22 | | | | |
| | Probe: CCCACGCCATCCGCCTGCTCCT | 68.9 | | | | |
| Tumor suppressor p53 | Forward: TAACAGTTCCTGCATGGGCGGC | 65.66 | NM_000546.5 | 121 | 1.96 | [32] |
| | Reverse: AGGACAGGCACAAACACGCACC | 66.01 | | | | |
| | Probe: CGGAGGCCCATCCTCACCATCATCA | 67.97 | | | | |
| Actin beta (*ACTB*) | Forward: CCTCGCCTTTGCCGA | 56.04 | NM_001101.3 | 171 | 2.23 | [33] |
| | Reverse: TGGTGCCTGGGGCG | 58.15 | | | | |
| | Probe: CCGCCGCCCGTCCACACCCGCC | 69.7 | | | | |

AgNPs diluted in DMEM/F12 medium showed a second peak between 550 and 560 nm. The absorption spectra of AgNPs incubated with HeLa and MCF-7 cell lines did not present any other modification. Incubation at 37°C and 5% $CO_2$ for 24 and 48 hours did not produce any significant change in the UV-visible spectra as compared to 0 hours of incubation.

*3.2. Cell Viability of HeLa and MCF-7 Cell Lines Exposed to Piton and Avocado AgNPs.* The MTT colorimetric assay was used to assess the *in vitro* cytotoxic effect of the two types of AgNPs on MCF-7 and HeLa cancer cell lines. Absorbance data were transformed to cell viability rates using (1). Untreated cells without exposure to AgNPs (control) represented 100% of cell viability. Results showed that MCF-7 cell viability rates decreased when piton and avocado AgNPs concentration increased. In comparison with the control, this dose-dependent cytotoxicity was statistically significant at the concentration of 50 $\mu$M ($p = 0.0203$) and 80 $\mu$M ($p = 0.0003$) for piton AgNPs, showing a cell viability decrease of approximately 16% and 25%, respectively (Figure 1(a)). The effect of avocado AgNPs on MCF-7 cell line was significant at 50 $\mu$M ($p = 0.0097$) and 80 $\mu$M ($p < 0.0001$) causing a decrease of 19% and 27% in cell viability, respectively (Figure 1(b)). Tukey's multiple comparison tests were used to determine which treatments were different from each other. The viability means of 1 $\mu$M and 80 $\mu$M treatments for both types of AgNPs showed a p-value of 0.0249 for piton AgNPs and 0.0371 for avocado AgNPs. Figures 2(a) and 2(b) show that the confidence intervals (95% confidence level) for the difference between the means of 1 $\mu$M and 80 $\mu$M do not contain zero. Consequently, a statistically significant difference for these means is supported. Conversely, HeLa cells treated with piton and avocado AgNPs in concentrations up to 50 $\mu$M did not show a statistically significant cytotoxic response ($p > 0.05$). The viability of treated HeLa cells remained similar to nonexposure control (Figures 1(c) and 1(d)), and no relevant differences were obtained with Tukey's multiple comparison tests and confidence intervals (data not shown).

*3.3. Relative Quantification of GST and p53 Genes.* *GST* and p53 genes are important in mounting cellular defense responses against harmful stimuli causing, for instance, DNA damage [34, 35]. We conducted *GST* and p53 relative mRNA expression analysis by qRT-PCR from RNA extracted from MCF-7 and HeLa cells exposed to *G. neuberthii* and *P. americana* AgNPs. Cells were treated with various concentrations of NPs (0, 40, 80, and 160 $\mu$M for MCF-7 cell line, and 0, 25, 50, and 100 $\mu$M for HeLa cell line) for 48 h, then RNAs were isolated, and qRT-PCR assays were conducted as described in Methods. Exposure of MCF-7 cells to 40 $\mu$M of *G. neuberthii* and *P. americana* AgNPs resulted in a statistically significant downregulation of p53 with relative expression levels of 0.527 ($p = 0.0499$) and 0.560 ($p = 0.0499$), respectively (Figure 3(a)). In HeLa cells, *G. neuberthii* AgNPs at 100 $\mu$M and *P. americana* AgNPs at 25 $\mu$M also decreased p53 expression (to a relative expression of 0.331, $p = 0.0499$ and 0.234, $p = 0.0062$, resp.) (Figure 3(b)). For both cell lines, *GST* gene expression was not significantly affected by AgNPs treatment at any tested concentration (Figures 3(a) and 3(b)). Although we found some AgNPs treatments to slightly higher *GST* expression levels, these results were not statistically relevant.

## 4. Discussion

There is a considerable amount of literature on naked silver nanoparticles that show their antimicrobial [23], anti-inflammatory [22], anticancer, antiangiogenic [38], antiviral [39–41], and antiproliferative [42] properties. However, toxicity evaluation of silver NPs in mammalian cells has shown adverse effects [43]. Several *in vitro* assays have reported that Zn and Fe nanoparticles induce oxidative stress, DNA damage, and apoptosis in human hepatoma cells,

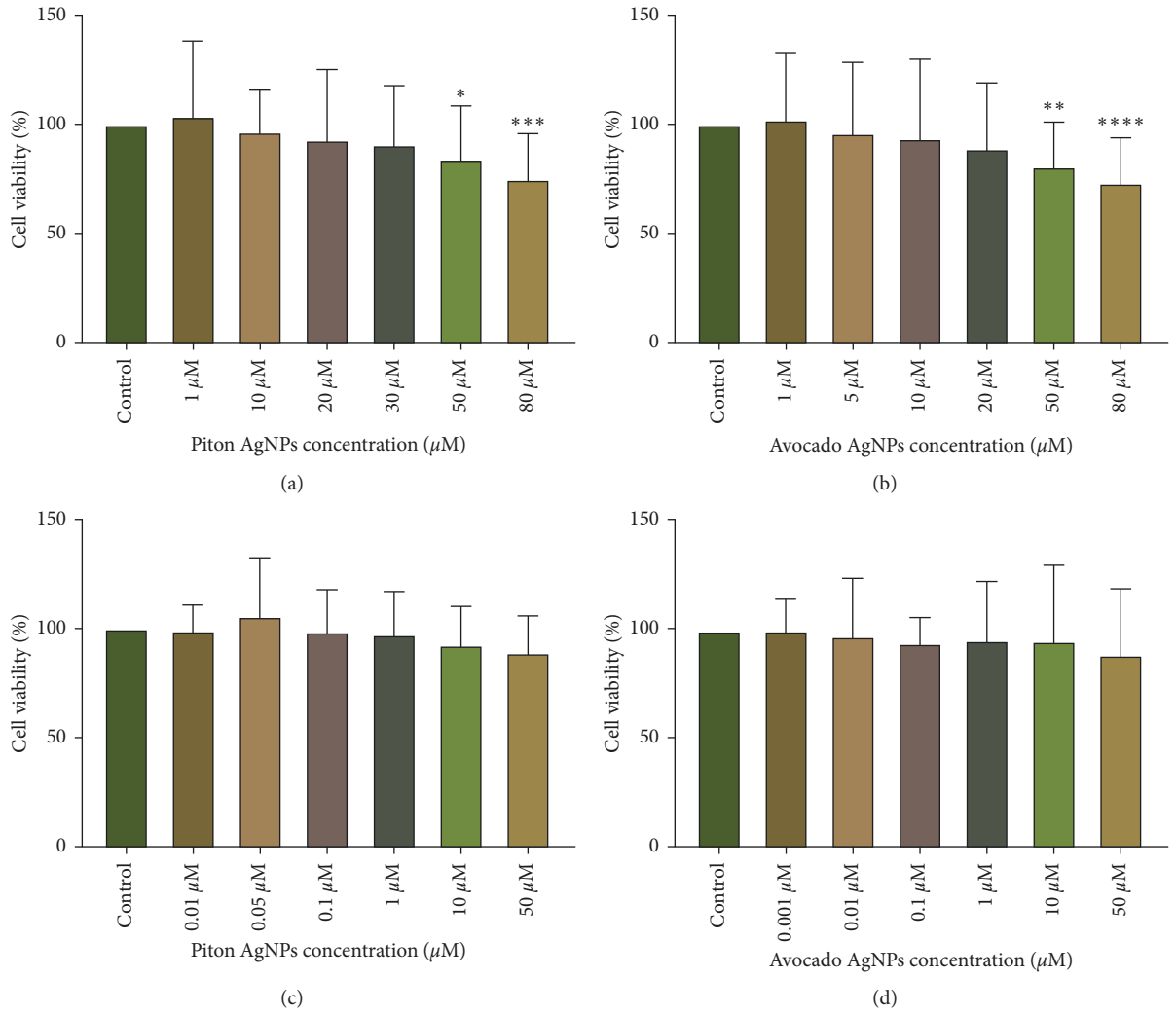


Figure 1: Viability of MCF-7 (a, b) and HeLa (c, d) cells treated with piton AgNPs or avocado AgNPs relative to viability of the control (untreated cells). Cells were treated with several concentrations of AgNPs for 48 hours. Data are presented as mean ± SD from three independent experiments ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, and $^{****}p \leq 0.0001$).

hepatocytes, and lymphocytes [44]. *In vivo* experiments on Crl:CD(SD)IGS BR 344 rats showed pulmonary inflammation and cytotoxicity following exposure to single-wall carbon nanotubes [45].

When studying organically coated nanoparticles (OC-NPs), it is important to understand their chemistry, dissolution rate, surface properties, and determine their physical properties to evaluate their *in vivo* and *in vitro* effects. For instance, OC-AgNPs interact with aqueous solutions and form Ag+ which leads to membrane and subcellular components damage [46]. However, it is essential to evaluate all the different coatings in order to explore new applications.

To the best of our knowledge, the present study is the first one that evaluates both *P. americana* and *G. neuberthii*-coated AgNPs biological *in vitro* effects on MCF-7 and HeLa cells. After bio-synthesized AgNPs physical characterization, stability under culture conditions was assessed using UV-Vis absorption spectrometry which provided information on the structural conformation of organic or inorganic elements in eluted solutions [47]. The principle of localized surface plasmon resonance (LSPR) states that when light interacts with conductive nanoparticles (i.e., AgNPs) which are smaller than the incident wavelength, the resultant electric field excites electrons and generates plasmon oscillations which are dependent on the composition, size, geometry, dielectric environment, and separation distance of NPs [48]. UV-Vis analysis of spectra of organically coated nanoparticles suspended in DI water for HeLa in our study did not show an absorbance peak due to the fact that it was highly diluted, whereas for MCF-7, a small peak was present at 400–420 nm. Stability assays on complete medium showed the presence of the previously described peak and a second one between 550 and 560 nm. Since AgNPs absorbance is in the range of 390–430 nm [49, 50], our results confirm the presence of nanoparticles. However, synthesized NPs lacked uniformity, suggesting that the obtained NPs have different shapes, sizes [49], and were not completely monodispersed

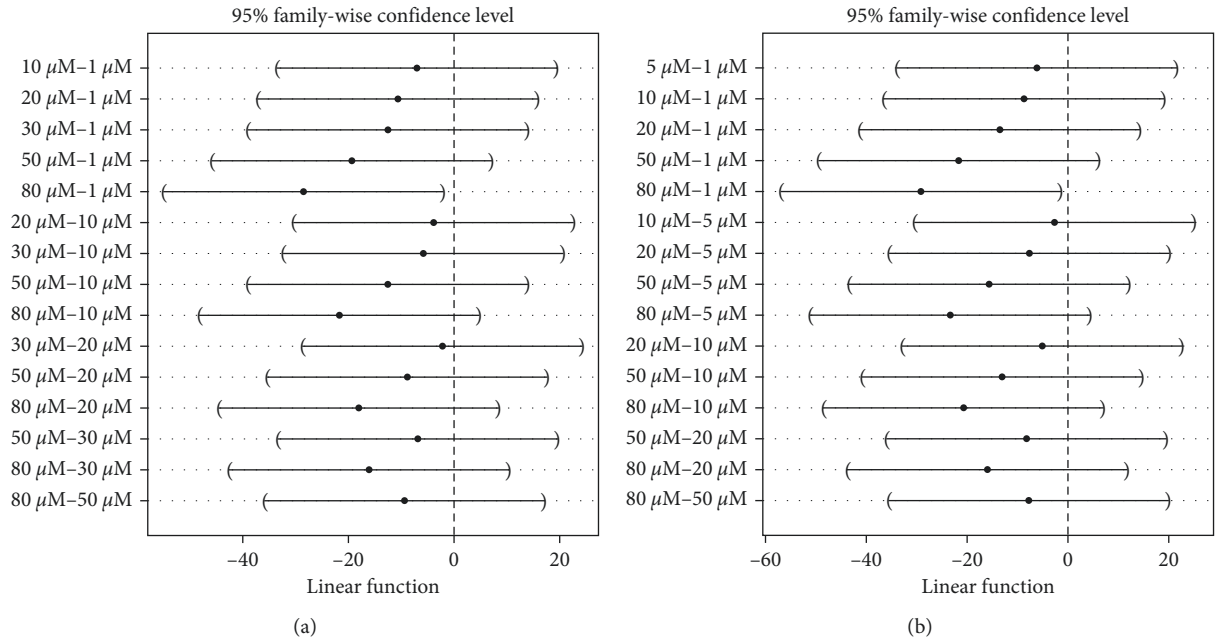


Figure 2: Interval plots for differences of means with 95% confidence levels for (a) piton AgNPs treatments and (b) avocado AgNPs treatments on MCF-7 cell line viability. The confidence intervals for the pairs of means that include zero represent that the differences are not statistically significant.

(agglomeration) [51]. The second peak clearly corresponded to DMEM, which absorbs at wavelengths from 440 to 560 nm depending on the solution's pH [52]. Although this peak corresponds to the growth medium, a related point to consider is the presence of diverse amino acids, growth factors, and FBS that might also contribute to cause AgNPs aggregation [53].

An important point to note is that HeLa cells showed a reduced cellular uptake in comparison with MCF-7 cells. Biosynthesized AgNPs in our study became aggregated, but endocytosis depends not only on aggregation status but also on multiple factors such as size, charge, surface coating, interactions with the culture media, and cell-specific uptake properties [54].

Gliga and collaborators studied the importance of agglomeration in the cellular uptake of coated AgNPs, concluding that the primary particle size is the most important factor that contributes to Ag+ release and subsequently cellular toxicity [55]. For instance, hydrophobically modified glycol CS (HGCS) nanoparticles were evaluated on HeLa cells where most of them were internalized by the non-destructive mechanism of micropinocytosis (used for agglomerated NPs) instead of the clathrin-mediated endocytosis route. These internalization pathways exhibit diverse intracellular behaviors and trigger different levels of cytotoxicity, including inhibition of lysosome degradation [56].

Cytotoxicity MTT assays showed a dose-dependent toxicity in the MCF-7 cell line with a statistically significant reduction in cell viability at concentrations of 50 $\mu$M and 80 $\mu$M for both nanoparticles. The cytotoxic effect found in the present study is in agreement with findings from other studies. For instance, green biosynthesized AgNPs (*Tanacetum vulgare*, phycocyanin) can exert a lethal effect on MCF-7 cells [57, 58]. Further analysis from Figure 1(a) (*G. neuberthii*) and Figure 1(b) (*P. americana*) in MCF-7 cells indicates a reduction of viability of up to 25% and 27%, respectively. However, high toxicity levels (above 50%) have been previously reported by Gurunathan et al. [59] using biologically synthesized AgNPs on the same cell line with toxicity being mediated via micropinocytosis and clathrin-dependent endocytosis [60]. However, the morphology, size, and surface chemical groups of nanoparticles affect the above mechanisms [61] and our bio-AgNPs lacked monodispersity. According to El-Naggar et al. [58], AgNPs action mechanisms involve the release of silver cations and further interaction with biomolecules such as DNA and proteins, affecting cell membrane integrity, lactate dehydrogenase (LDH) levels and mitochondrial permeability and leading finally to oxidative stress and apoptosis.

In contrast, HeLa cells MTT viability assessments (Figures 1(c) and 1(d)) suggest that non-monodispersed nanoparticles interfered with $Ag^+$ ion release. Furthermore, the agglomeration/aggregation state of AgNPs influences cellular uptake depending mainly on cell types and their properties, as being reported by Lankoff et al. [62] whose results are similar to ours. To sum up, HeLa cells exhibited a reduced uptake in the presence of agglomerated AgNPs, conversely to MCF-7 cells, which showed a higher uptake rate for this type of NPs.

An interesting aspect to address is the interaction among AgNPs coating with cell membranes which are negatively charged in mammalian cells at pH = 7. Electric variations of this layer affect the transport of substrates, as reported by Dobrzyńska et al., who described electrical membrane variations of MCF-7 cells according to their media pHs [63]. The authors showed a shift of the isoelectric point at low pH

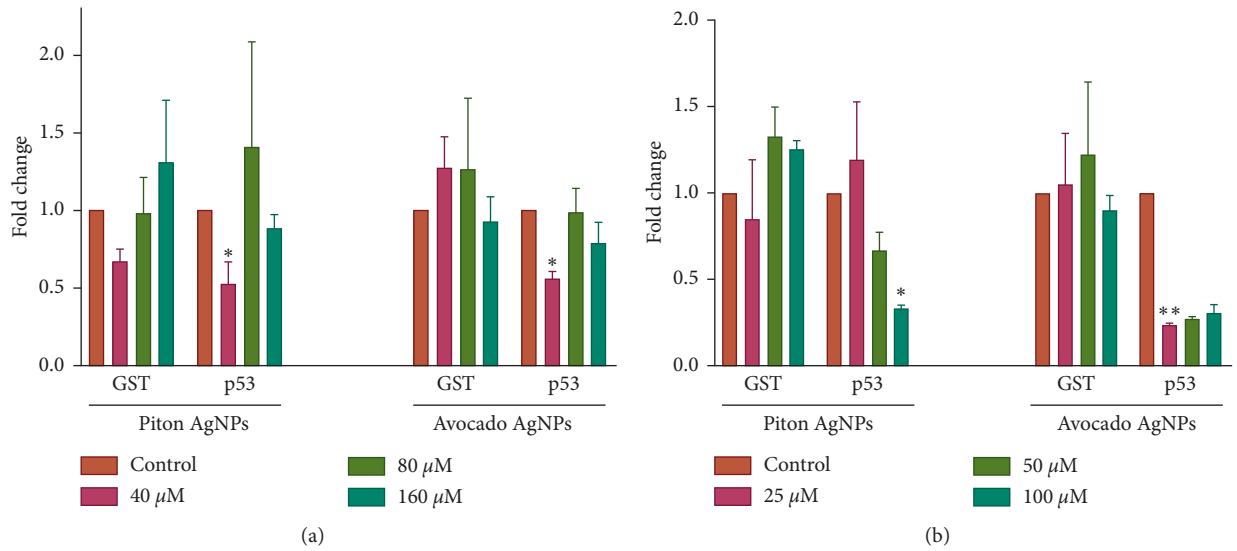


Figure 3: Relative expression of *GST* and p53 genes in (a) MCF-7 and (b) HeLa cells treated with piton and avocado AgNPs. Actin beta was used as the reference gene for data normalization. Untreated cells were used as calibrators to calculate the fold changes. Data are presented as mean ± SD from three independent experiments ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, and $^{****}p \leq 0.0001$).

values and a positive charge was evidenced at low pH, with negative charge at high pH. In the case of HeLa cells, a study by Warren and Payne found that nanoparticles do not permeabilize the membrane but allow depolarization by increasing potassium channels, which leads to stabilization of the resting membrane potential [64]. In the present study, different responses in the studied cell lines suggest that intrinsic differences affect the uptake and internalization of nanoparticles. Cell culture components (media and serum) affect nanoparticles and cause aggregation, which might affect the intracellular behavior of NPs but not necessarily inhibit their effect [63]. This is due to the fact that NPs cellular uptake is always mediated by a biocorona, which affects NPs biodistribution, activation, and interaction with cell surface receptors. As reported by Asharani et al., NPs size affects binding and activation of membrane receptors and gene expression in cancer cell lines [42].

Although AgNPs cytotoxicity has been found in several studies [14,66–69], the molecular mechanisms involved are not completely understood [16]. AgNPs may trigger oxidative stress by reactive oxygen species (ROS) production [70, 71] causing a variety of intracellular responses and alterations in antioxidant systems [72], which finally could lead to apoptosis or necrosis [73].

Recent studies have reported a remarkable increase in expression levels of *GST* in different *in vitro* [16] [31] and *in vivo* [74, 75] models treated with AgNPs, showing that *GST* is an important factor to balance intracellular oxidative status [76]. We found, however, that exposure to biosynthesized AgNPs did not elicit statistically significant variations in gene expression in treated cancer cells after 48 hours of incubation/exposure. This lack of response regarding *GST* expression might be explained by the presence of bioactive compounds that may counteract the formation of intracellular free radicals. It has been previously reported by Owolabi et al. [77] that phytochemicals such as quercetin have strong antioxidant activity in extracts from *P. americana* leaves. Alva et al. [27] found that *G. neuberthii* fruit contains monounsaturated and polyunsaturated fatty acids, which are known for their capacity to scavenge free radicals, for example, superoxide [78]. However, further experiments are required to confirm the presence of antioxidants in the nanoparticles and inside the cells.

An analysis of the expression levels of genes coding for detoxifying enzymes by Aueviriyavit et al. [79] found that *GST* expression levels remained unchanged after Caco-2 cells were exposed to AgNPs, despite the fact that expression levels of other stress-responsive genes were significantly affected. Kumaran et al. [31] found that *GST* expression in MCF-7 cells became altered after 24 hours of exposure to NPs rather than 48 hours. This phenomenon was explained by a higher NPs: cell ratio at 24 hours in comparison with the proliferation rate at 48 hours, where intracellular amounts of NPs decrease, resulting in less cytotoxicity.

Another interesting study showed that a possible mechanism of death induced by NPs in cell lines is p53-mediated apoptosis, mechanism that depends, among other things, on the size of the nanomaterial studied [80–82]. Blanco et al. suggested that p53 expression might be downregulated when smaller NPs and longer incubation times are assayed [14]. In the present study, p53 gene expression decreased significantly at different concentrations after 48 hours of incubation with *G. neuberthii* and *P. americana* AgNPs (around 40 nm in diameter), which is in agreement with the study by Blanco et al. We found that *P. americana* AgNPs induced a statistically significant p53 downregulation after 48 hours of exposure to concentrations of 25 $\mu$M and 40 $\mu$M. Throughout, *G. neuberthii* AgNPs exhibited the same pattern for 40 $\mu$M and 100 $\mu$M. Our findings are in agreement with other studies, such as a recent one by Asharani et al. in which the authors found p53 to become downregulated along with low levels of p21 protein in normal human lung cells (IMR-90) and human

brain cancer cells (U251) after exposure to AgNPs [83]. Zhang et al. [65] found that AgNPs (400$\mu$g/ml) in several cancer cell lines induced downregulation of p53 expression and cell cycle arrest at S/G2/M phases.

In our study, HeLa cells line showed a p53 significant downregulation at 25 $\mu$M and 100 $\mu$M. However, at any concentration, relevant toxicity was absent. This finding might be explained by the role of p53 as a crucial regulator of cell proliferation and genome stability. p53 induces the expression of p21 transcription factor in presence of stress signals in order to trigger cell cycle arrest and senescence [36]. In primary tumors, however, p53 might be mutated with inhibition of its normal function as a result or might become degraded by ubiquitin-protein ligase targeting. Engeland found that p53 activation leads to cell cycle arrest by the p53-DREAM pathway. This network involves the downregulation of several genes including the major checkpoint p53 [37]

A correlation between mRNA p53 levels and cell viability was found in a study by Kovács and collaborators. The authors found a decrease in osteosarcoma U-2 OS cell viability when they were treated with AgNPs, with p53 significantly upregulated [15]. However, there are other primary mechanisms that are involved in apoptosis triggering, for example, mitochondrial stress. Our findings are in agreement with Kovács' study, as we observed a downregulation of p53 gene while cell viability was not significantly affected, thus supporting the notion that p53 downregulation might induce proliferation arrest.

In summary, MCF-7 cells underwent cycle arrest when exposed to our biosynthesized AgNPs, while HeLa cells were not affected. This finding might be of use when designing novel therapeutic strategies in cancer, specifically in breast cancer.

## 5. Conclusions

In the present study, we tested the cytotoxic/antiproliferative effect of biosynthesized AgNPs from *P. americana* and *G. neuberthii* on MCF-7 and HeLa cell lines. Expression of p53 and *GST* genes was also assessed for NPs' apoptosis triggering and oxidative stress modulation properties, respectively. Biosynthesized AgNPs were toxic in a concentration-dependent manner on MCF-7 cells but not on HeLa cells. *GST* expression was not affected, while p53 was downregulated in treated MCF-7 cells. Our findings demonstrate a net cytotoxic effect of both AgNPs on MCF-7 cells with a possible small apoptotic population and a large cell population going into proliferation arrest. While there is a clear need of mechanistic studies on the above cell responses, synthesized AgNPs might be useful when designing future therapeutic applications in breast cancer.

## Data Availability

The data supporting this research article are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Acknowledgments

The authors are thankful to Dr. Brajesh Kumar for provision of biosynthesized NPs and to Dr. Rachid Seqqat for valuable help with preliminary experiments. This work was supported by Universidad de las Fuerzas Armadas ESPE Research Grant 2014-PIT-044 (to Marcelo Grijalva).

## Supplementary Materials

UV-visible spectra analysis of piton/avocado AgNPs under biological conditions. (*Supplementary Materials*)

## References

[1] L. A. Torre, F. Islami, R. L. Siegel, E. M. Ward, and A. Jemal, "Global cancer in women: burden and trends," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 26, no. 4, pp. 444–457, 2017.

[2] Y. J. Ho, T. C. Wang, C. H. Fan, and C. K. Yeh, "Current progress in antivascular tumor therapy," *Drug Discovery Today*, vol. 22, no. 10, pp. 1503–1515, 2017.

[3] X.-F. Zhang, Z.-G. Liu, W. Shen, and S. Gurunathan, "Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches," *International Journal of Molecular Sciences*, vol. 17, no. 9, p. 1534, 2016.

[4] J. Elgqvist, "Nanoparticles as theranostic vehicles in experimental and clinical applications-focus on prostate and breast cancer," *International Journal of Molecular Sciences*, vol. 18, no. 5, p. 1102, 2017.

[5] F. Ordikhani, M. Erdem Arslan, R. Marcelo et al., "Drug delivery approaches for the treatment of cervical cancer," *Pharmaceutics*, vol. 8, no. 3, p. 23, 2016.

[6] K. Juarez-Moreno, E. B. Gonzalez, N. Giron-Vazquez et al., "Comparison of cytotoxicity and genotoxicity effects of silver nanoparticles on human cervix and breast cancer cell lines," *Human & Experimental Toxicology*, vol. 36, no. 9, pp. 931–948, 2017.

[7] D. Sahu, G. M. Kannan, M. Tailang, and R. Vijayaraghavan, "*In vitro* cytotoxicity of nanoparticles: a comparison between particle size and cell type," *Journal of Nanoscience*, vol. 2016, Article ID 4023852, 9 pages, 2016.

[8] S. Iravani, H. Korbekandi, S. V. Mirmohammadi, and B. Zolfaghari, "Synthesis of silver nanoparticles: chemical, physical and biological methods," *Research in Pharmaceutical Sciences*, vol. 9, no. 6, pp. 385–406, 2014.

[9] M. Grijalva, M. J. Vallejo, L. Salazar, J. Camacho, and B. Kumar, "Cytotoxic and antiproliferative effects of nanomaterialson cancer cell lines: a review," in A. Gomes, Ed., *Unraveling the Safety Profile of Nanoscale Particles and Materials,* InTech, Rijeka, Croatia, 2018.

[10] A. M. Yassin, N. M. El-Deeb, A. M. Metwaly, G. F. El Fawal, M. M. Radwan, and E. E. Hafez, "Induction of apoptosis in human cancer cells through extrinsic and intrinsic pathways by *Balanites aegyptiaca Furostanol saponins* and saponin-coated silvernanoparticles," *Applied biochemistry and Biotechnology*, vol. 182, no. 4, pp. 1675–1693, 2017.

[11] S. Dehghanizade, J. Arasteh, and A. Mirzaie, "Green synthesis of silver nanoparticles using *Anthemis atropatana* extract: characterization and in vitro biological activities," *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 46, no. 1, pp. 160–168, 2018.

[12] P. P. Banerjee, A. Bandyopadhyay, S. N. Harsha et al., "*Mentha arvensis* (Linn.)-mediated green silver nanoparticles trigger caspase 9-dependent cell death in MCF7 and MDA-MB-231 cells," *Breast Cancer*, vol. 9, pp. 265–278, 2017.

[13] Y.-J. Choi, J.-H. Park, J. W. Han et al., "Differential cytotoxic potential of silver nanoparticles in human ovarian cancer cells and ovarian cancer stem cells," *International Journal of Molecular Sciences*, vol. 17, no. 12, p. 2077, 2016.

[14] J. Blanco, D. Lafuente, M. Gomez, T. Garcia, J. L. Domingo, and D. J. Sanchez, "Polyvinyl pyrrolidone-coated silver nanoparticles in a human lung cancer cells: time- and dose-dependent influence over p53 and caspase-3 protein expression and epigenetic effects," *Archives of Toxicology*, vol. 91, no. 2, pp. 651–666, 2017.

[15] D. Kovács, N. Igaz, C. Keskeny et al., "Silver nanoparticles defeat p53-positive and p53-negative osteosarcoma cells by triggering mitochondrial stress and apoptosis," *Scientific Reports*, vol. 6, no. 1, article 27902, 2016.

[16] M. Zuberek, D. Wojciechowska, D. Krzyzanowski, S. Meczynska-Wielgosz, M. Kruszewski, and A. Grzelak, "Glucose availability determines silver nanoparticles toxicity in HepG2," *Journal of Nanobiotechnology*, vol. 13, no. 1, p. 72, 2015.

[17] C. Licciardello, N. D'Agostino, A. Traini, G. R. Recupero, L. Frusciante, and M. L. Chiusano, "Characterization of the glutathione S-transferase gene family through ESTs and expression analyses within common and pigmented cultivars of *Citrus sinensis* (L.) Osbeck," *BMC Plant Biology*, vol. 14, no. 1, p. 39, 2014.

[18] D. Dabas, R. M. Shegog, G. R. Ziegler, and J. D. Lambert, "Avocado (*Persea americana*) seed as a source of bioactive phytochemicals," *Current Pharmaceutical Design*, vol. 19, no. 34, pp. 6133–6140, 2013.

[19] M. Yasir, S. Das, and M. D. Kharya, "The phytochemical and pharmacological profile of *Persea americana* Mill," *Pharmacognosy Reviews*, vol. 4, no. 7, pp. 77–84, 2010.

[20] D. Rodriguez-Sanchez, C. Silva-Platas, R. P. Rojo et al., "Activity-guided identification of acetogenins as novel lipophilic antioxidants present in avocado pulp (*Persea americana*)," *Journal of Chromatography B*, vol. 942–943, pp. 37–45, 2013.

[21] A. Kosińska, M. Karamać, I. Estrella, T. Hernández, B. Bartolomé, and G. A. Dykes, "Phenolic compound profiles and antioxidant capacity of *Persea americana* mill. peels and seeds of two varieties," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 18, pp. 4613–4619, 2012.

[22] P. Anitha and P. Sakthivel, "Synthesis and characterization of silver nanoparticles using *Persea americana* (Avocado) and its anti-inflammatory effects on human blood cells," *International Journal of Pharmaceutical Sciences Review and Research*, vol. 35, no. 2, pp. 173–177, 2015.

[23] S. P Vinay, N. Chandrashekar, and C. P. Chandrappa, "Silver nanoparticles: synthesized by leaves extract of Avocado and their antibacterial activity," *International Journal of Engineering Development and Research*, vol. 5, no. 2, pp. 1608–1613, 2017.

[24] J. León, *Botánica de los Cultivos Tropicales*, 2000.

[25] M. S. Gachet, J. S. Lecaro, M. Kaiser et al., "Assessment of antiprotozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis," *Journal of Ethnopharmacology*, vol. 128, no. 1, pp. 184–197, 2010.

[26] M. M. Grandtner and J. Chevrette, *Dictionary of Trees: Nomenclature, Taxonomy and Ecology*, Elsevier Science, Vol. 2, Elsevier Science, New York, NY, USA, 2013.

[27] A. Alva, O. Vásquez, R. Cunibertti, A. Castillo, and W. Guerra, "Extracción y Caracterización de Ácidos Grasos de la especie *Grias neuberthii* Macht (Sachamango)," *Revista Amazónica de Investigación Alimentaria*, vol. 2, no. 1, pp. 103–106, 2002.

[28] P. Vásquez-Ocmín, S. Cojean, E. Rengifo et al., "Antiprotozoal activity of medicinal plants used by Iquitos-Nauta road communities in Loreto (Peru)," *Journal of Ethnopharmacology*, vol. 210, pp. 372–385, 2017.

[29] S. Barua, P. P. Banerjee, A. Sadhu et al., "Silver nanoparticles as antibacterial and anticancer materials against human breast, cervical and oral cancer cells," *Journal of Nanoscience and Nanotechnology*, vol. 17, no. 2, pp. 968–976, 2017.

[30] M. W. Pfaffl, "A new mathematical model for relative quantification in real-time RT-PCR," *Nucleic Acids Research*, vol. 29, no. 9, p. e45, 2001.

[31] R. S. Kumaran, Y.-K. Choi, H. J. Kim, and K. J. Kim, "Quantitation of oxidative stress gene expression in MCF-7 human cell lines treated with water-dispersible CuO nanoparticles," *Applied Biochemistry and Biotechnology*, vol. 173, no. 3, pp. 731–740, 2014.

[32] Y. C. Chew, G. Adhikary, G. M. Wilson, W. Xu, and R. L. Eckert, "Sulforaphane induction of p21(Cip1) cyclin-dependent kinase inhibitor expression requires p53 and Sp1 Transcription Factors and Is p53-dependent," *Journal of Biological Chemistry*, vol. 287, no. 20, pp. 16168–16178, 2012.

[33] K. Andrea Kreß, *The Tax Protein of Human T Cell Lymphotropic Virus Type 1 as a Multifunctional Oncoprotein*, 2011.

[34] Z. Lei, T. Liu, X. Li, X. Xu, and D. Fan, "Contribution of glutathione S-transferase gene polymorphisms to development of skin cancer," *International Journal of Clinical and Experimental Medicine*, vol. 8, no. 1, pp. 377–389, 2015.

[35] T. Ozaki and A. Nakagawara, "Role of p53 in cell death and human cancers," *Cancers*, vol. 3, no. 1, pp. 994–1013, 2011.

[36] R. A. Shamanna, M. Hoque, T. Pe'ery, and M. B. Mathews, "Induction of p53, p21 and apoptosis by silencing the NF90/NF45 complex in human papilloma virus-transformed cervical carcinoma cells," *Oncogene*, vol. 32, p. 5176, 2012.

[37] K. Engeland, "Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM," *Cell Death and Differentiation*, vol. 25, no. 1, pp. 114–132, 2018.

[38] S. Gurunathan, K.-J. Lee, K. Kalishwaralal, S. Sheikpranbabu, R. Vaidyanathan, and S. H. Eom, "Antiangiogenic properties of silver nanoparticles," *Biomaterials*, vol. 30, no. 31, pp. 6341–6350, 2009.

[39] H. R. Kim, D. Y. Shin, Y. J. Park, C. W. Park, S. M. Oh, and K. H. Chung, "Silver nanoparticles induce p53-mediated apoptosis in human bronchial epithelial (BEAS-2B) cells," *Journal of Toxicological Sciences*, vol. 39, no. 3, pp. 401–412, 2014.

[40] L. Sun, A. K. Singh, K. Vig, S. R. Pillai, and S. R. Singh, "Silver nanoparticles inhibit replication of respiratory syncytial virus," *Journal of Biomedical Nanotechnology*, vol. 4, no. 2, pp. 149–158, 2008.

[41] D. Baram-Pinto, S. Shukla, N. Perkas, A. Gedanken, and R. Sarid, "Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate," *Bioconjugate Chemistry*, vol. 20, no. 8, pp. 1497–1502, 2009.

[42] P. V. Asharani, M. P. Hande, and S. Valiyaveettil, "Anti-proliferative activity of silver nanoparticles," *BMC Cell Biology*, vol. 10, p. 65, 2009.

[43] M. Ahamed, M. S. AlSalhi, and M. K. J. Siddiqui, "Silver nanoparticle applications and human health," *Clinica Chimica Acta*, vol. 411, no. 23-24, pp. 1841–1848, 2010.

[44] V. Kumar, N. Sharma, and S. S. Maitra, "In vitro and in vivo toxicity assessment of nanoparticles," *International Nano Letters*, vol. 7, no. 4, pp. 243–256, 2017.

[45] D. B. Warheit, B. R. Laurence, K. L. Reed, D. H. Roach, G. A. M. Reynolds, and T. R. Webb, "Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats," *Toxicological Sciences*, vol. 77, no. 1, pp. 117–125, 2004.

[46] V. K. Sharma, K. M. Siskova, R. Zboril, and J. L. Gardea-Torresdey, "Organic-coated silver nanoparticles in biological and environmental conditions: fate, stability and toxicity," *Advances in Colloid and Interface Science*, vol. 204, pp. 15–34, 2014.

[47] V. Williamson and S. Kumar, "Spectroscopy of organic compounds," *Department of Chemistry*, vol. 66, pp. 1–36, 2006.

[48] E. Petryayeva and U. J. Krull, "Localized surface plasmon resonance: Nanostructures, bioassays and biosensing-a review," *Analytica Chimica Acta*, vol. 706, no. 1, pp. 8–24, 2011.

[49] A. Mubayi, S. Chatterji, P. M. Rai, and G. Watal, "Evidence based green synthesis of nanoparticles," *Advanced Materials Letters*, vol. 3, no. 6, pp. 519–525, 2012.

[50] A. Saxena, R. M. Tripathi, F. Zafar, and P. Singh, "Green synthesis of silver nanoparticles using aqueous solution of *Ficus benghalensis* leaf extract and characterization of their antibacterial activity," *Materials Letters*, vol. 67, no. 1, pp. 91–94, 2012.

[51] A. N. Geraldes, A. A. da Silva, J. Leal et al., "Green nanotechnology from plant extracts : synthesis and characterization of gold nanoparticles," *Advanced in Nanoparticles*, vol. 5, no. 3, pp. 176–185, 2016.

[52] I. Tasset, F. J. Medina, J. Peña et al., "Olfactory bulbectomy induced oxidative and cell damage in rat: protective effect of melatonin," *Physiological Research*, vol. 59, no. 1, pp. 105–112, 2010.

[53] A. C. Sabuncu, J. Grubbs, S. Qian, T. M. Abdel-Fattah, M. W. Stacey, and A. Beskok, "Probing nanoparticle interactions in cell culture media," *Colloids and Surfaces B: Biointerfaces*, vol. 95, pp. 96–102, 2012.

[54] S. Salatin and A. Yari Khosroushahi, "Overviews on the cellular uptake mechanism of polysaccharide colloidal nanoparticles," *Journal of Cellular and Molecular Medicine*, vol. 21, no. 9, pp. 1668–1686, 2017.

[55] A. R. Gliga, S. Skoglund, I. O. Wallinder, B. Fadeel, and H. L. Karlsson, "Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release," *Particle and Fibre Toxicology*, vol. 11, no. 1, p. 11, 2014.

[56] S. Park, S. J. Lee, H. Chung et al., "Cellular uptake pathway and drug release characteristics of drug-encapsulated glycol chitosan nanoparticles in live cells," *Microscopy Research and Technique*, vol. 73, no. 9, pp. 857–865, 2010.

[57] Q. Liu and H. Jiang, "In vitro cytotoxicity of *Tanacetum vulgare* mediated silver nanoparticles against breast cancer (MCF-7) cell lines," *Biomedical Research*, vol. 28, no. 3, pp. 1354–1358, 2017.

[58] N. E.-A. El-Naggar, M. H. Hussein, and A. A. El-Sawah, "Bio-fabrication of silver nanoparticles by phycocyanin, characterization, in vitro anticancer activity against breast cancer cell line and in vivo cytotxicity," *Scientific Reports*, vol. 7, no. 1, article 10844, 2017.

[59] S. Gurunathan, J. W. Han, A. A. Dayem et al., "Green synthesis of anisotropic silver nanoparticles and its potential cytotoxicity in human breast cancer cells (MCF-7)," *Journal of Industrial and Engineering Chemistry*, vol. 19, no. 5, pp. 1600–1605, 2013.

[60] M. Milić, G. Leitinger, I. Pavičić et al., "Cellular uptake and toxicity effects of silver nanoparticles in mammalian kidney cells," *Journal of Applied Toxicology*, vol. 35, no. 6, pp. 581–592, 2014.

[61] A. Nel, T. Xia, L. Mädler, and N. Li, "Toxic potential of materials at the nanolevel," *Science*, vol. 311, no. 5761, pp. 622–627, 2006.

[62] A. Lankoff, W. J. Sandberg, A. Wegierek-Ciuk et al., "The effect of agglomeration state of silver and titanium dioxide nanoparticles on cellular response of HepG2, A549 and THP-1 cells," *Toxicology Letters*, vol. 208, no. 3, pp. 197–213, 2012.

[63] I. Dobrzyńska, E. Skrzydlewska, and Z. A. Figaszewski, "Changes in electric properties of human breast cancer cells," *Journal of Membrane Biology*, vol. 246, no. 2, pp. 161–166, 2013.

[64] E. A.K. Warren and C. K. Payne, "Cellular binding of nanoparticles disrupts the membrane potential," *RSC Advances*, vol. 5, no. 18, pp. 13660–13666, 2015.

[65] X.-F. Zhang, W. Shen, and S. Gurunathan, "Silver nanoparticle-mediated cellular responses in various cell lines: an in vitro model," *International Journal of Molecular Sciences*, vol. 17, no. 10, p. 1603, 2016.

[66] M. Wypij, J. Czarnecka, M. Swiecimska, H. Dahm, M. Rai, and P. Golinska, "Synthesis, characterization and evaluation of antimicrobial and cytotoxic activities of biogenic silver nanoparticles synthesized from *Streptomyces xinghaiensis* OF1 strain," *World Journal of Microbiology & Biotechnology*, vol. 34, no. 2, p. 23, 2018.

[67] A. P. Kanjikar, A. L. Hugar, and R. L. Londonkar, "Characterization of phyto-nanoparticles from *Ficus krishnae* for their antibacterial and anticancer activities," *Drug Development and Industrial Pharmacy*, vol. 44, no. 3, pp. 377–384, 2018.

[68] L. Qiu, J.-W. Li, C.-Y. Hong, and C.-Y. Pan, "Silver nanoparticles covered with pH-sensitive camptothecin-loaded polymer prodrugs: switchable fluorescence 'off' or 'on' and drug delivery dynamics in living cells," *ACS Applied Materials & Interfaces*, vol. 9, no. 46, pp. 40887–40897, 2017.

[69] J. S. Park, E.-Y. Ahn, and Y. Park, "Asymmetric dumbbell-shaped silver nanoparticles and spherical gold nanoparticles green-synthesized by mangosteen (*Garcinia mangostana*) pericarp waste extracts," *International Journal of Nanomedicine*, vol. 12, pp. 6895–6908, 2017.

[70] X.-F. Zhang and S. Gurunathan, "Combination of salinomycin and silver nanoparticles enhances apoptosis and autophagy in human ovarian cancer cells: an effective anticancer therapy," *International Journal of Nanomedicine*, vol. 11, pp. 3655–3675, 2016.

[71] J. W. Han, S. Gurunathan, J.-K. Jeong et al., "Oxidative stress mediated cytotoxicity of biologically synthesized silver nanoparticles in human lung epithelial adenocarcinoma cell line," *Nanoscale Research Letters*, vol. 9, no. 1, p. 459, 2014.

[72] D. B. Zorov, M. Juhaszova, and S. J. Sollott, "Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release," *Physiological Reviews*, vol. 94, no. 3, pp. 909–950, 2014.

[73] T. Zhang, L. Wang, Q. Chen, and C. Chen, "Cytotoxic potential of silver nanoparticles," *Yonsei Medical Journal*, vol. 55, no. 2, pp. 283–291, 2014.

[74] S. Saddick, M. Afifi, and O. A. A. Zinada, "Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii*," *Saudi Journal of Biological Sciences*, vol. 24, no. 7, pp. 1672–1678, 2017.

[75] T. Coccini, R. Gornati, F. Rossi et al., "Gene expression changes in rat liver and testes after lung instillation of a low dose of silver nanoparticles," *Journal of Nanomedicine & Nanotechnology*, vol. 5, no. 5, 2014.

[76] P. M.G. Nair and J. Choi, "Identification, characterization and expression profiles of *Chironomus riparius* glutathione S-transferase (GST) genes in response to cadmium and silver nanoparticles exposure," *Aquatic Toxicology*, vol. 101, no. 3-4, pp. 550–560, 2011.

[77] M. A. Owolabi, H. A B Coker, and S. Jaja, "Bioactivity of the phytoconstituents of the leaves of *Persea americana*," *Journal of Medicinal Plants Research*, vol. 4, no. 12, 2010.

[78] D. Richard, K. Kefi, U. Barbe, P. Bausero, and F. Visioli, "Polyunsaturated fatty acids as antioxidants," *Pharmacological Research*, vol. 57, no. 6, pp. 451–455, 2008.

[79] S. Aueviriyavit, D. Phummiratch, and R. Maniratanachote, "Mechanistic study on the biological effects of silver and gold nanoparticles in Caco-2 cells–induction of the Nrf2/HO-1 pathway by high concentrations of silver nanoparticles," *Toxicology Letters*, vol. 224, no. 1, pp. 73–83, 2014.

[80] S. Park, H. H. Park, S. Y. Kim, S. J. Kim, K. Woo, and G. Ko, "Antiviral properties of silver nanoparticles on a magnetic hybrid colloid," *Applied and Environmental Microbiology*, vol. 80, no. 8, pp. 2343–2350, 2014.

[81] S. R. Satapathy, P. Mohapatra, R. Preet et al., "Silver-based nanoparticles induce apoptosis in human colon cancer cells mediated through p53," *Nanomedicine*, vol. 8, no. 8, pp. 1307–1322, 2013.

[82] W. Wang, C. Zeng, Y. Feng et al., "The size-dependent effects of silica nanoparticles on endothelial cell apoptosis through activating the p53-caspase pathway," *Environmental Pollution*, vol. 233, pp. 218–225, 2018.

[83] P. V. Asharani, S. Sethu, H. K. Lim, G. Balaji, S. Valiyaveettil, and M. P. Hande, "Differential regulation of intracellular factors mediating cell cycle, DNA repair and inflammation following exposure to silver nanoparticles in human cells," *Genome Integrity*, vol. 3, no. 1, pp. 1–14, 2012.