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Green synthesis of colloidal gold nanoparticles using latex from *Hevea brasiliensis* and evaluation of their in vitro cytotoxicity and genotoxicity

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Abstract: Latex extracted from *Hevea brasiliensis* tree has been used as a green alternative for preparing gold nanoparticles (Au NPs); however, no study evaluating the cytotoxic and genotoxic potential of Au NPs synthesised using *H. brasiliensis* has been published. The present study aimed to synthesise and characterise colloidal Au NPs using latex from *H. brasiliensis* and to evaluate their in vitro cytotoxicity and genotoxicity. Ideal conditions for the green synthesis of Au NPs were studied. *In vitro* cytotoxicity and genotoxicity of Au NPs in CHO-K1 cells was also evaluated. Our findings indicated that the ideal synthesis conditions of pH, temperature, reduction time, and concentrations of latex and $HAuCl_4$ were 7.0, 85°C, 120 min, 3.3 mg/mL, and 5.0 mmol/L, respectively. $LC50_{24\,h}$ of Au NPs was 119.164 ± 5.31 μg/mL. Lowest concentration of Au NPs tested presented minimal cytotoxicity and genotoxicity. However, high concentrations of Au NPs promoted DNA damage and cell death via apoptosis. On the basis of these findings, the authors optimised the use of an aqueous solution of *H. brasiliensis* latex as a reducing/stabilising agent for the green synthesis of Au NPs. Low concentrations of these NPs are biocompatible in normal cell types, suggesting that these NPs may be used in biological applications.

1 Introduction

The development of green chemistry techniques has experienced explosive growth over the past decades. Increasingly, researchers are shifting their attention towards the use of organic (natural) reducing/stabilising agents for the use in the synthesis of gold nanoparticles (Au NPs). Extracts from plants [1, 2], fruits [3–5], leaves [6, 7], and flowers [8–10] as well as microbiological compounds extracted from marine algae [11] and fungi [12–14] are being used. The use of such natural compounds, instead of chemical solvents, minimises the generation of chemical waste.

Studies involving the green synthesis of NPs using live plants from the Euphorbiaceae family have been reported [15]. Among these, a study has reported that latex extracted from *Hevea brasiliensis* tree can be an alternative material for preparing NPs. Bakar *et al.* (2007) [16], Guidelli *et al.* (2011) [17], and Danna *et al.* (2016) [18] synthesised silver NPs using liquid natural rubber obtained from *H. brasiliensis* latex. In addition to the synthesis of silver NPs, recent studies have reported the synthesis of Au NPs using latex extracted from *H. brasiliensis*. Cabrera *et al.* (2013) [19] reported the *in situ* synthesis of Au NPs using solid natural rubber as a reducing/stabilising agent. These natural agents have been successfully shown to inhibit the proliferation of *Leishmania brasiliensis* promastigotes in vitro [20]. They also serve as chemical sensors in surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS) [21]. Tao *et al.* (2015) [22] and Tao *et al.* (2018) [23] synthesised Au NPs on the surface of spherical natural rubber particles obtained from the latex of *H. brasiliensis*; these were used to fabricate a flexible and effective SERS substrate.

The biomedical applications of Au NPs in biosensorics, immunoassays, drug delivery, and optical bioimaging have been extensively studied [24]. However, for these applications to be realised, the synthesised nanomaterials need to be biocompatible. *In vitro* models are widely used to test the biocompatibility of materials to assess whether they can cause injurious effects on biological systems. Cytotoxicity and genotoxicity tests are used to evaluate the biocompatibility of a material in vitro [25]. Several studies have evaluated the in vitro cytotoxicity, genotoxicity, and anticancer activity of Au NPs produced through green synthesis [26]. Gold nanoparticles synthesised from phytolatex are promising candidates as components to enhance sun protection factor in sunscreen formulations [27].

This study aimed to synthesise and characterise colloidal Au NPs using latex from *H. brasiliensis* (Euphorbiaceae) as a reducing/stabilising agent and to evaluate their in vitro cytotoxicity and genotoxicity. Different synthesis parameters, including pH, temperature, reduction time, and the concentrations of both latex and gold salt aqueous solutions, were evaluated. To our knowledge, this was the first study to evaluate the in vitro cytotoxic and genotoxic potentials of Au NPs synthesised using *H. brasiliensis*.

2 Materials and methods

2.1 Latex collection from H. brasiliensis

Latex was collected from different *H. brasiliensis* rubber trees (RRIM 600 clone), which belongs to the Euphorbiaceae family, from an experimental farm in Indiana, Sao Paulo, Brazil. The latex was stabilised with 2% ammonium hydroxide and stored under refrigeration (5°C).

2.2 Optimisation of the synthesis of Au NPs

Latex stabilised with 2% ammonium hydroxide was diluted in Milli-Q^{TM} water (18.2 mΩ), according to the methods reported by Bar *et al.* (2009) [28] and Guidelli *et al.* [17]. Gold (III) chloride trihydrate ($HAuCl_4.3H_2O$) was purchased from *Synth* Company.

To evaluate the effect of latex concentration on Au NP synthesis, different proportions of latex (0.2, 0.5, 1.0, and 2.0 mL) were diluted in 300 mL water and mixed with $HAuCl_4$ suspension (2:1, v/v) to prepare $NRL_{0.65}$–Au (0.65 mg/mL), $NRL_{1.6}$–Au (1.6 mg/mL), $NRL_{3.3}$–Au (3.3 mg/mL), and $NRL_{6.5}$–Au (6.5 mg/mL) samples, respectively. Synthesis of Au NPs was performed at pH

7.0 and 85°C with a reduction time of 120 min and 5.0 mmol/L $HAuCl_4$.

To evaluate the effect of pH on Au NP synthesis, pH of NRL-Au suspension was set at 6.0, 7.0, 8.0, and 9.0 using NaOH; pH below 5.0 was not tested because the latex coagulated in solutions with a pH below 5.0 (data not presented). Tests were carried out at 85°C, with 5.0 mmol/L of $HAuCl_4$, reduction time of 120 min, and the $NRL_{3.3}$ latex suspension. The optimal reduction time was evaluated from 5 to 240 min under constant stirring. The pH was controlled at 7.0, maintaining the temperature of synthesis at 85°C and the concentration of $HAuCl_4$ at 5.0 mmol/L. In addition, the $NRL_{3.3}$ latex suspension was used.

To evaluate the effect of metal ion concentration on Au NP synthesis, Au NPs were formed with a direct reaction of latex in an $HAuCl_4$ suspension at concentrations of 1.0, 3.0, 5.0, and 10.0 mmol/L. The $NRL_{3.3}$ latex suspension was used at pH 7.0, temperature of 85°C, and reduction time of 120 min.

To evaluate the effect of temperature, the synthesis of Au NPs was carried out at 60, 70, and 85°C using the $NRL_{3.3}$ latex suspension, 120 min of reduction time, and 5.0 mmol/L of $HAuCl_4$ at pH 7.0. The temperatures used for these experiments were defined as previously reported [29], where it was shown that temperatures between 70°C and 90°C favor fast reduction of Au NPs.

2.3 Characterisation of Au NPs

UV-Vis plasmon absorption data were obtained using a Varian spectrophotometer (Cary 50) from 190 to 800 nm at a scan rate of 400 nm s^{-1}. Transmission electron microscopy (TEM) images of Au NPs synthesised using latex were obtained using an FEI Tecnai G2 F20 microscope operating at 200 kV. The distribution of Au NP diameter was evaluated using the ImageJ software. TEM samples were prepared through dropwise addition of an Au NP suspension onto carbon-coated copper TEM grids. Fourier-transform infrared (FTIR) spectra were recorded using a Bruker Vector 22 spectrometer with a 4 cm^{-1} spectral resolution and 32 scans, in a wavelength range of 400–4000 cm^{-1}. *X*-ray diffraction (XRD) analysis (Shimadzu XRD-6000) was obtained using a with Cu Kα1 ($\lambda = 1.5406$ Å) and Cu Kα2 ($\lambda = 1.5444$ Å), at 40 kV and 30 mA, with 0.02° step, scanning speed of 2°/min and angular range $2\theta =$ 5° to 90°.

2.4 Biological evaluation

The in vitro cytotoxic and genotoxic potentials of the synthesised NPs were tested under the following conditions: pH 7.0, 85°C, reduction time of 120 min, and $HAuCl_4$ concentration of 5.0 mmol/L, using the $NRL_{3.3}$ latex suspension. Chinese hamster ovarian (CHO-K1) cells were cultured in 10 mL Dulbecco's Modified Eagle's Medium/F10 Ham (1:1) supplemented with 10% foetal bovine serum in 25-cm^2 cell culture flasks. The cells were maintained in an incubator without CO_2 at 37°C.

2.4.1 Cytotoxic potential of Au NPs: Cells were seeded on a transparent 24-well plate at a density of 2.0×10^5 cells per well. The cells were incubated for 24, 48, or 96 h with culture medium only [negative control (NC)] or with different concentrations of NPs: 5.0 E9, 5.0 E10, 5.0 E11 particles/mL in the same volume of culture medium, corresponding to 0.0368, 0.368, and 3.68 ng/mL, respectively. Number of NPs was calculated according to methods described by Haiss *et al.* (2007) [30] and TN801 (2008) [31]. The cytotoxic potential of Au NPs was evaluated using the MTT reduction method, as described by Mosmann (1983) [32]. The absorbance obtained for NC cells was considered to represent 100% cell viability. The viability of cells treated with the other samples was determined by the following formula: CVK = [(AK–AB)/(ANC–AB)] × 100 where: CVK = cell viability of the cells exposed to Au NPs; AK = absorbance of cells exposed to Au NPs; ANC = absorbance of the negative control cells; AB = absorbance of the blank.

To determine the lethal concentration that kills 50% of the cells (LC50) within 24 h of exposure, the cells (2.0×10^5 cells per well) were exposed to gold nanoparticles at concentrations of 1 μg/ml to 200 μg/ml.

Using the MTT Assay, the cytotoxic potential of the latex was tested at a concentration of 3.3 mg/mL, which was the concentration used for the formation of Au NPs that had their cytotoxicity tested. The cytotoxicity of the Intermediate Fraction (FI), which was extracted from the latex by centrifugation, was also evaluated using the MTT Assay with exposure times of 24, 48, and 96 h.

2.4.2 Evaluation of DNA damage: To evaluate DNA damage in cells exposed to Au NPs, an alkaline version of the comet assay was performed. For this assay, cells were seeded at a density of 5.0×10^5 cells per well in a 12-well plate, and then exposed to different concentrations of NPs or PBS (NC) for a period of 24, 48, or 96 h. Next, an equal volume of culture medium was added to each well. After the exposure period, cell suspensions were used to prepare slides for the comet assay, as described by Singh et al. (1988) [33]. The slides were then stained with DAPI solution (1 mg/mL) and visualised by fluorescence microscopy. One hundred cells were counted per slide, and DNA damage was classified into four categories according to the migration of DNA fragments, as described by Kobayashi et al. (1995) [34].

2.4.3 Morphological detection of apoptosis and necrosis: Cells were seeded at a density of 2.0×10^5 cells per well in a 12-well plate and exposed to different concentrations of NPs or PBS (NC) for a period of 24, 48, or 96 h. An equal volume of culture medium was added to each well. After the exposure period, the cell suspension was mixed with Hoechst 33342 (1000 μg/mL) and propidium iodide (1000 μg/mL). Slides were prepared and visualised by fluorescence microscopy. Two hundred cells per slide were analysed as apoptotic, necrotic, or normal according to the following criteria: (1) normal cells: blue nucleus; (2) apoptotic cells: blue nucleus with apoptotic bodies; and (3) necrotic cells: red nucleus.

2.4.4 Statistical analysis: Biological results were compared by parametric analysis of variance using the Student–Newman–Keuls method or the non-parametric Kruskal–Wallis test, on the basis of data distribution (normality and homogeneity of variance). Values of $p < 0.05$ were considered significant.

3 Results and discussion

3.1 Effect of latex concentration

As shown in Fig. 1, an increase in absorption intensity was noted as latex volume increased from 0.2 to 1.0 mL. Differences in plasmonic bandwidth were observed, whereby the higher the latex concentration, the narrower was the plasmonic bandwidth. The results of the investigation of latex concentration showed that latex at different concentrations (0.65, 1.6, and 3.3 mg/mL) could be used to synthesise Au NPs. This was shown by the formation of a plasmonic band between the wavelengths 525 and 554 nm, which are similar to those reported in the literature. Particle size distribution increased as latex concentration increased up to 3.3 mg/mL, and as plasmonic band narrowed. In our previous study, the authors synthesised gold nanoparticles using NR membranes (solid) that release rubber and non-rubber compounds [19]. Functional non-rubber groups as complex carbonyl (esters, ketones, and aldehydes) that can be derived from proteins (even denatured), as well as primary amides or C = O of carboxylic dimer acid (–COOH), are suggested as reducing/stablish agents for use in the synthesis process of gold nanoparticles [35]. Other groups containing CH- or CH_2-, for example terpenoids, mainly found on an isoprene structure are also suggested to be involved, owing to the polymer's hydrolysis in water and to the α-terminal groups of mono- or diphosphate compounds linked with the phospholipids of rubber [36]. Tao *et al.* (2015) [22] suggested that hydroxyl groups are responsible in reducing Au nanoparticles, and free amine

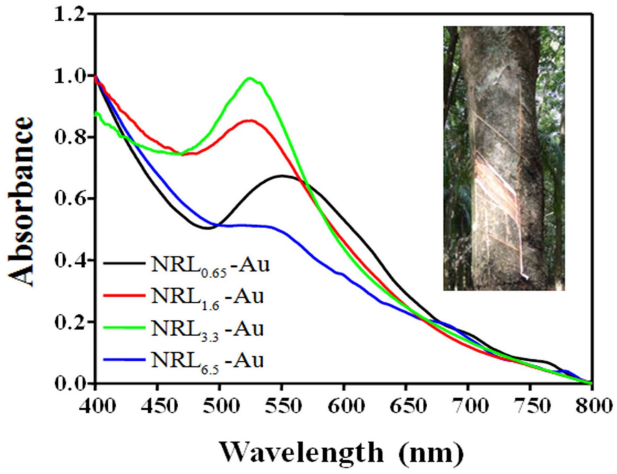

Fig. 1 *UV-Vis spectroscopy (normalised) evaluating the effect of latex volume on the synthesis of Au NPs*

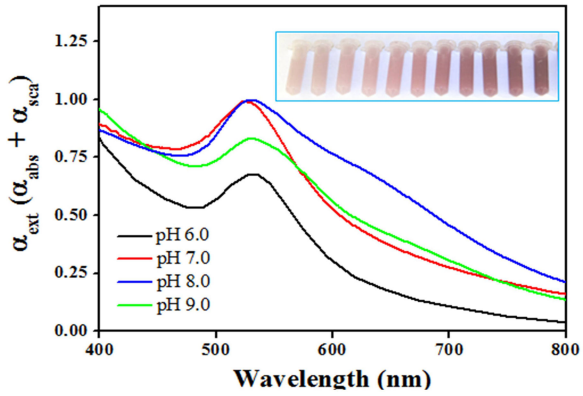

Fig. 2 *UV-Vis spectroscopy (normalised) evaluating the effect of pH on the synthesis of Au NPs*

groups could bind to the nanoparticle surface in order to stabilise it. Green synthesis of metal nanoparticles based on plants extracts has been attributed the reducing/stabilise agents to sugars, terpenoids, flavonoids, alkaloids, phenolic compounds, amino acids, and compounds from proteins, such as peptides, cysteine, and free amines [28, 37–48].

In the present study, a limit of concentration was reached because of a decrease in plasmon absorption at a concentration of 2.0 mL. This was attributed to the high concentration of latex, which was not consumed in the reducing process; this made the evaluation of nanoparticles difficult. According to the results of this study, the ideal volume of latex was 3.3 mg/mL because at this volume, Au NPs with a low size distribution were obtained.

3.2 Effect of pH

The surface plasmon resonance band of Au NPs appeared at ~530 nm in all pH tested (Fig. 2). The results obtained by TEM confirmed and complemented the results obtained by UV-Vis spectrometry. Fig. 3 shows TEM images evaluating the influence of pH during synthesis on the morphology (size and shape) of the Au NPs obtained. Spherical nanoparticles were found in all high-pH tested. Synthesis at pH 6.0 (Fig. 4*a*) resulted in average sizes of 6.0, 18.0, and 37.0 nm were observed. At pH 7.0, a narrow distribution of particles (with an average size of approximately 9.0 nm) was observed, and smaller particles were synthesised at this pH condition ((Fig. 4*b*). Increasing the pH to 8.0 increased the size of the Au NPs synthesised (mostly at 30.0 nm), which were spherical, showing sizes at between 20 and 40 nm (Fig. 4*c*). Finally, NPs with a wide range of sizes (6.0 to 30.0 nm) were obtained when the synthesis was performed at pH 9.0 (Fig. 4*d*). Thus, pH 7.0 was defined as the optimal pH for Au NP synthesis because this pH condition resulted in NPs with smaller particles size. Similar to the results of the present study, smaller gold nanoparticles were synthesised using black cardamom extract when the pH of the solution is at or ~7.0 [49].

3.3 Effect of reduction time

A ruby-red Au NPs suspension was obtained after mixing $HAuCl_4$ for 5 min with the latex solution. In other studies, this colour change was also observed when gold chloride solution was mixed with latex [27, 50]. The results showed that absorbance progressively increased as reduction time increased, leading to a plateau or saturation at 120 min (Fig. 5). A reduction time of 120 min was required for $NRL_{3.3}$ to efficiently reduce 5.0 mmol/L of $HAuCl_4$ to Au NPs (Fig. 4). Das *et al.* (2011) [29] reported that aqueous extracts of *Calotropis procera* latex require 20 min to efficiently reduce $HAuCl_4$ (1.0 mmol/L) to Au NPs. Borase *et al.* (2014) [27] observed that the absorbance of Au NPs colloid synthesised using latex from *Jatropha gossypiifolia* increased as incubation time increased.

3.4 Effect of metal ion concentration

Using various concentrations of metal ion, the authors revealed that plasmon absorption intensity increased as $HAuCl_4$ concentration increased from 1.0 to 5.0 mmol/L, reaching a saturation limit at 10.0 mmol/L (Fig. 6). The green synthesis of Au NPs is mainly defined by stoichiometry, which is common in the formation of colloidal particles. $HAuCl_4$ at 5.0 mmol/L was found to be the optimal concentration.

3.5 Effect of temperature

The rate of Au NP formation increased as temperature increased (Fig. 7), owing to faster reaction kinetics as temperature increases. Temperature did not markedly influence Au NP synthesis using aqueous extracts of *C. procera* latex, as reported by Das *et al.* (2009) [29]. Cabrera *et al.* (2013) [19] found that the optimal annealing temperature of the natural rubber membranes in the preparation of Au NPs colloid should be between 80°C and 120°C. From these results, the authors identified the optimal temperature as 85°C.

3.6 FTIR spectra

Peaks were shifted or detected at 929, 1049, and, 1329 cm^{-1}, related to carbon ligand from natural rubber structure. New peaks were observed following Au NP synthesis at 696, 1399, and 1623 cm^{-1} mainly related to nitrogen groups. The identification of each peak is provided in Table 1. The authors noticed that compounds containing oxygen and nitrogen atoms were mainly affected, which suggested the reducing agent and stabilise the nanoparticles, respectively. Terpenoids and flavonoids seems to be responsible for the reducing and stabilising properties of the latex because terpenoids have alcohols, aldehydes, amines, carboxylic acids, and ketones as functional groups. The peak detected at 3353 cm^{-1} could be related to the hydroxyl functional group in alcohols and phenolic compounds, whereas the band at 1637 cm^{-1} can be assigned to the amide I band (residue of the proteins) (Figs. 8 and 9).

3.7 XRD pattern

Fig. 10 shows a representative XRD pattern of the gold nanoparticles synthesised by *H. brasiliensis* latex. The diffractogram of the sample showed five diffraction peaks, referring to the planes (111), (200), (220), (311), and (222) [51], respectively. The peaks/planes obtained are very similar to the characteristic peaks/planes of the standard gold metal, comparing crystal forms using data from JCPDS-ICDD 2-1095. The presence of halo $2\theta = 19°$ in the diffraction profile indicates the low crystallinity of the latex/polymer [52].

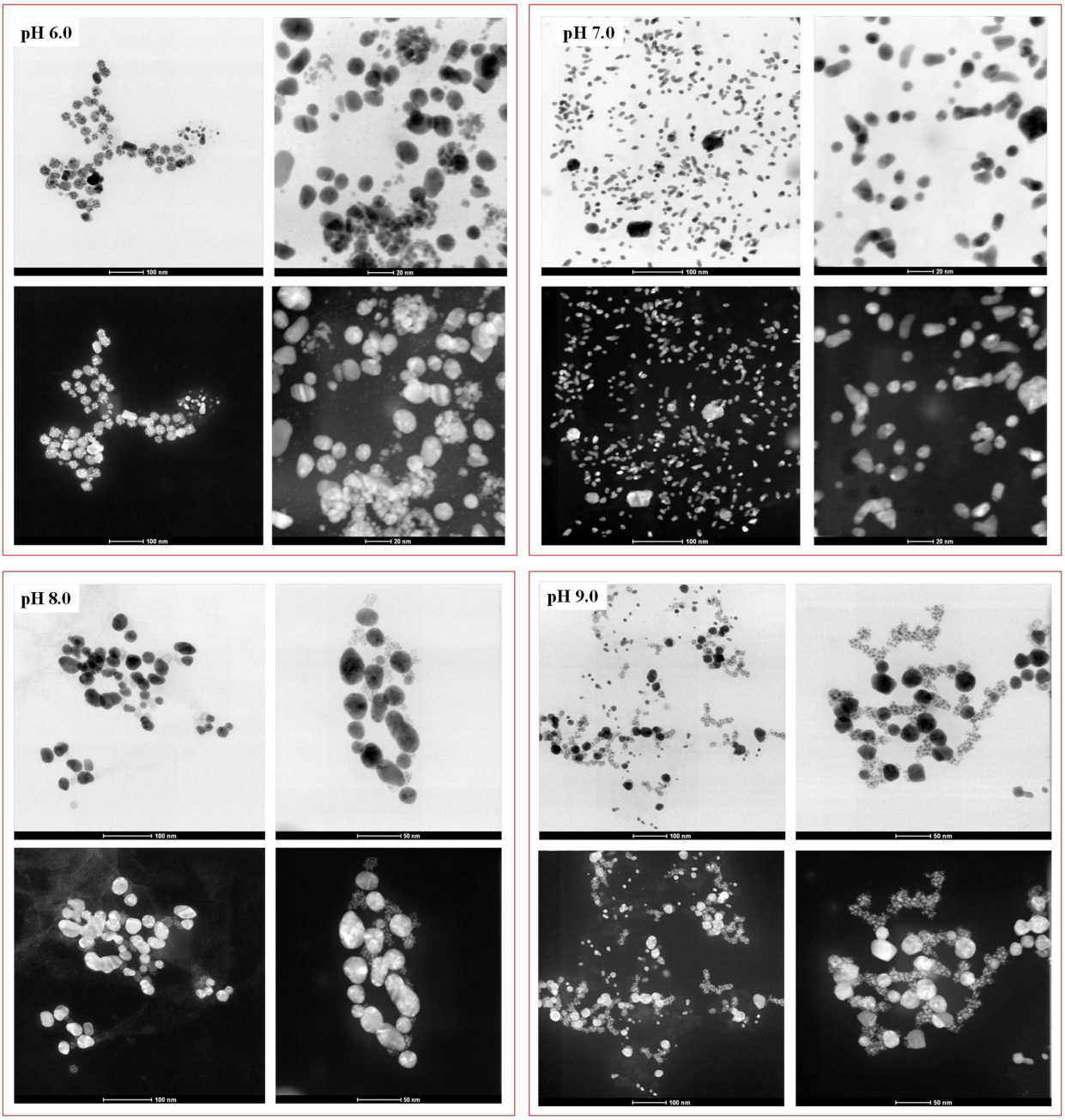

Fig. 3 *TEM images indicating the effect of pH on the size and shape of Au NPs, considering pH 6.0, 7.0, 8.0, and 9.0*

3.8 Cytotoxic potential

On the basis of the characterisation of Au NPs, and the variations observed with different parameters, the optimal conditions for the formation of colloidal Au NPs using *H. brasiliensis* latex as a reducing agent were defined as follows: pH 7.0, 85°C, reduction time of 120 min, $HAuCl_4$ concentration of 5.0 mmol/L, and latex concentration of $NRL_{3.3}$. Toxicity and genotoxicity tests are required to evaluate the safety of NPs. Thus, toxicological tests were carried out with the Au NPs synthesised under these conditions.

The results of the MTT assay revealed that the Au NPs, at almost all concentrations and exposure periods tested, were non-toxic to CHO-K1 cells (Fig. 11). The results indicated that the viability of CHO-K1 cells exposed to Au NPs at 5.0 E9, 5.0 E10, and 5.0 E11 particles/mL were not significantly different than that of NC cells at 24 and 48 h. Danna *et al.* (2016) [18] found that silver NPs synthesised from natural rubber membrane prepared with *H. brasiliensi*s latex presented low cytotoxicity to CHO-K1 cells after 24 h of exposure. Valodkar *et al.* (2011a) [44] found that silver and copper NPs synthesised with latex from *Euphorbia nivulia*, which also belongs to the Euphorbiaceae family, at nanomolar concentrations presented no cytotoxicity towards CHO-K1 cells after 72 h of exposure.

In the present study, a reduction in the viability of CHO-K1 cells (∼20%) was observed with Au NPs at 5.0 E11 particles/mL (3.6 ng/mL) and at the longest exposure period (96 h) (Fig. 11). Mishra *et al.* (2013) [53] found that, at a concentration of 2.5 ng/mL, gold nanoparticles (size range of 10–60 nm with near spherical morphology) synthesised using the extract of *Hibiscus sabdariffa* caused a decrease in viability of ∼30 and 95% in 293 normal cells and U87 GBM malignant cells, respectively, after 48 h of exposure. Gold nanoparticles (range of 8–42 nm) synthesised using *Torreya nucifera* at a concentration of 1 ng/mL showed cytotoxicity of 14.85% to 3T3-L1 cells after exposure for 24 h [54]. Valodkar *et al.* (2011b) [55] showed that silver NPs synthesised using latex from *E. nivulia* were cytotoxic to A549 cells in a dose-dependent manner and that this cytotoxicity was related to the internalisation of these NPs and generation of oxidative stress. The relationship between size of gold nanoparticles and cell damage has been quite prominent in the scientific literature. Hanan *et al.* (2018) [26] showed a correlation

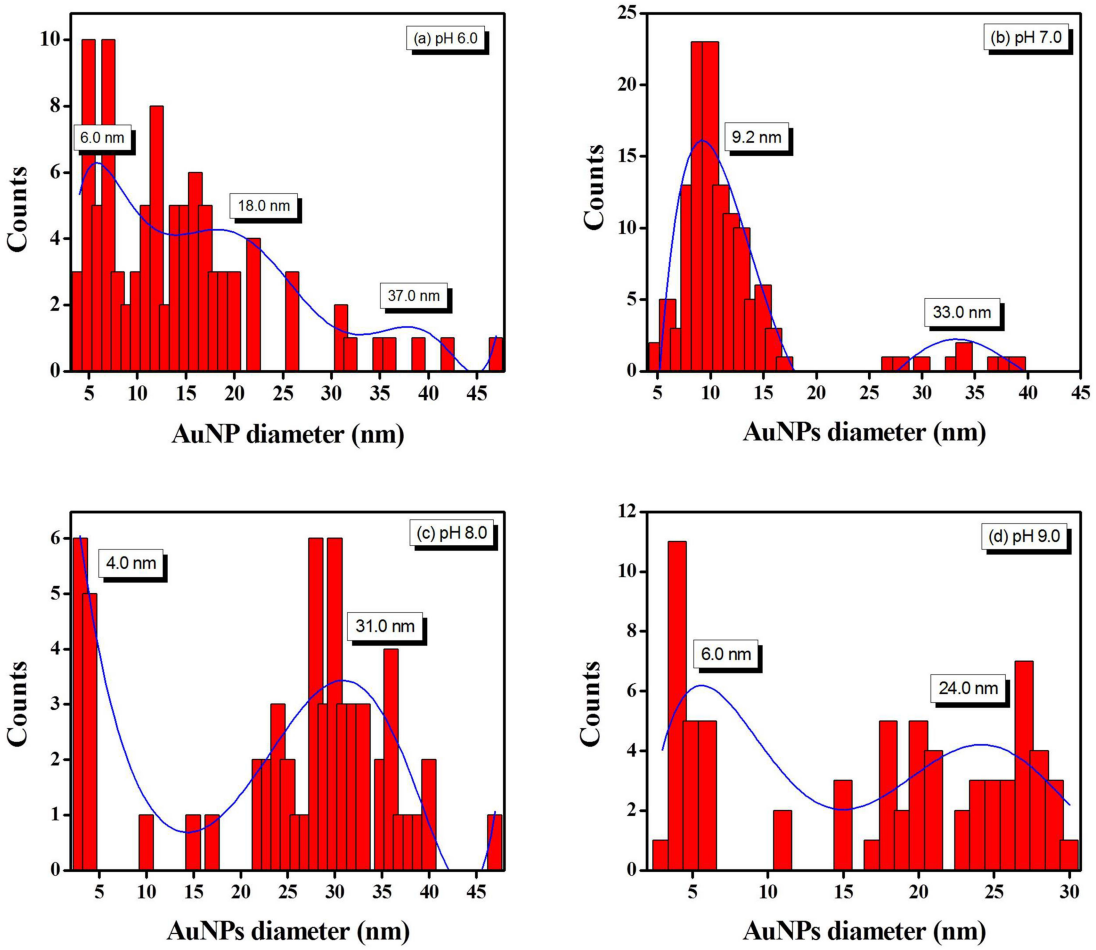

Fig. 4 *Histogram and polynomial fit thereof showing the diameter distribution of AuNPs evaluated by Transmission electronic microscopy (TEM), considering the effect of pH on the synthesis of gold nanoparticles, for pH 6.0, 7.0, 8.0 and 9.0*

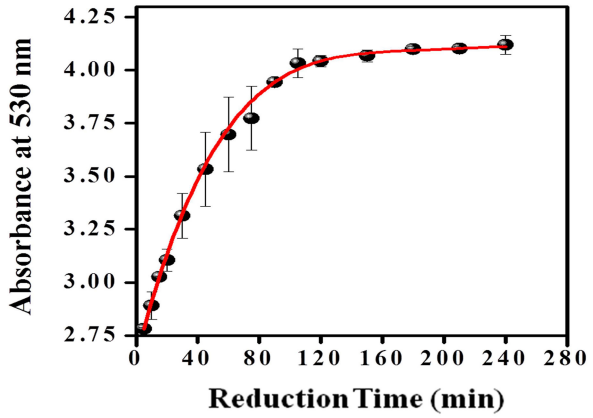

Fig. 5 *UV-Vis spectroscopy evaluating the effect of reduction time on the synthesis of Au NPs*

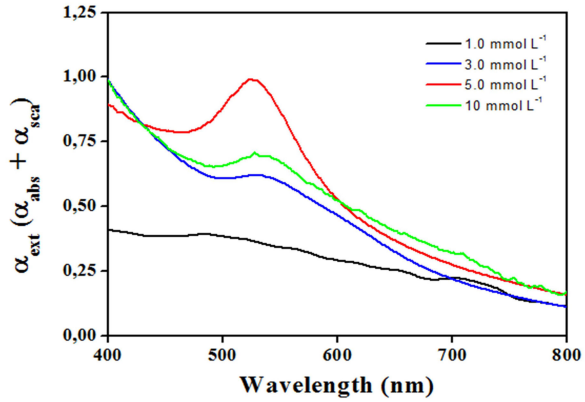

Fig. 6 *UV-Vis spectroscopy (normalised) evaluating the effect of $HAuCl_4$ concentration on the synthesis of Au NPs*

between the average size of plant-based metallic nanoparticles and cytotoxicity, and they found that cytotoxicity is inversely proportional to size, that is, smaller-sized gold nanoparticles are highly toxic compared to larger sized gold nanoparticles. In the present study, gold nanoparticles synthesised with latex from *H. brasiliensis* have an average size of ~9.0 nm, and this may have contributed to the cytotoxic potential found in the high concentrations of nanoparticles. Small gold nanoparticles can easily be internalised into cells; the greater the amount of nanoparticle incorporated into the cells, the greater the concentration, leading to increased cytotoxicity [56].

The results of the cytotoxicity testing of Au NPs at different concentrations to CHO-K1 cells showed a dose-dependent response; it was found that the LC50 for the 24-h exposure was 119.164 ± 5.31 µg/mL (Fig. 12).

The relationship between gold nanoparticles surface and cell damage is well reported in scientific literature. According to Tao *et al.* (2015) [22] the surfaces of gold nanoparticles synthesised with latex from *H. brasiliensis* are surrounded by proteins from the non-rubber fraction of *H. brasiliensis* latex. Furuya *et al.* (2017a) [57] and Furuya *et al.* (2017b) [58] showed that, among the three latex fractions obtained after centrifugation, that is, rubber component, intermediate phase, and sediment, the presence of the non-rubber constituents dominantly affects cytotoxicity. By analysing the results of the cytotoxicity test performed using the MTT Assay, as presented in Fig. 13, the authors were able to verify that there was difference in the viability of the cells exposed to latex at 3.3 mg/mL compared to NC. A decrease in viability of 15% was observed after 24 h of exposure. Natural rubber (NR) latex contains 4–5% weight of non-rubber constituents, such as protein, lipids,

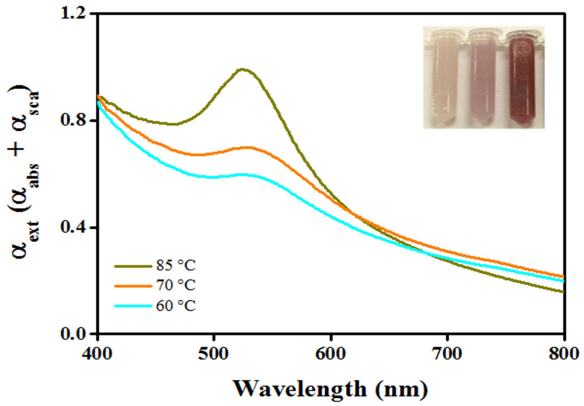

Fig. 7 *UV-Vis spectroscopy (normalised) evaluating the effect of temperature on the synthesis of Au NPs*

Table 1 Fourier transform infrared (FTIR) peaks and related assignment according to the literature

Experimental, cm^{-1}	Attributed
696	N-H, C=O or C-H out-of-plane bending
929	Stretching C-C
1049	Stretching C–O of ether
1329	C–N aromatic amines or C-O
1399	N-O2 Nitro groups, C-H alkenes or CH3 alkanes
1623	(NH)C=O primary amide
3353	N-H stretching or OH- contribution (O–H alcohols or phenols)

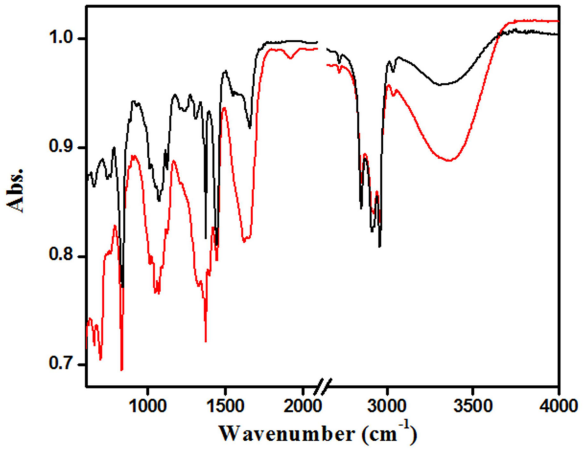

Fig. 8 *Absorbance spectra of latex (black) and AuNPs (red) determined by Fourier Transform Infrared (FTIR). Synthesis carried out using pH 7.0, 85°C, reduction time of 120 min, $HAuCl_4$ concentration of 5.0 mmol/L, and latex concentration of $NRL_{3.3}$*

carbohydrates, and sugar. Considering this data, NRL3.3 contains a maximum of 165 µg/mL of non-rubber constituents. Our cytotoxicity results showed that the intermediate fraction (FI) at this concentration did not alter the viability of the exposed cells at exposure times of 24, 48, and 96 h. Taken together, it was not possible to establish a relationship between gold nanoparticles surrounded by non-rubber constituents as proteins and increased cytotoxicity.

3.9 Morphological detection of apoptosis and necrosis

Compared to the NC, Au NPs at the three concentrations tested did not affect the percentage of apoptotic and necrotic cells at 24 and 48 h. However, compared to the NC, NPs at a concentration of 5.0 E11 increased the percentage of apoptotic cells, but not of necrotic cells at 96 h (Fig. 14).

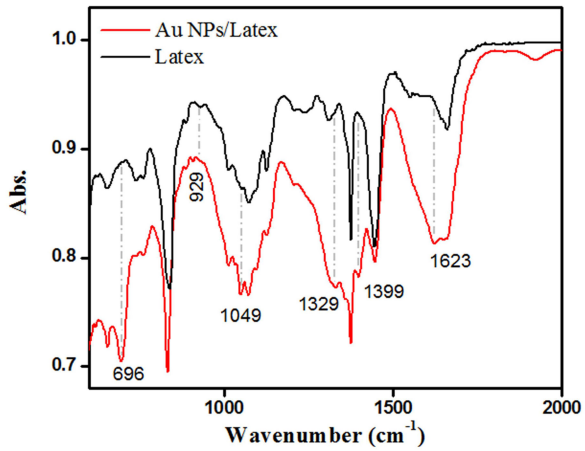

Fig. 9 *Absorbance spectra of latex and Au NPs determined by Fourier Transform Infrared (FTIR), highlighting peaks*

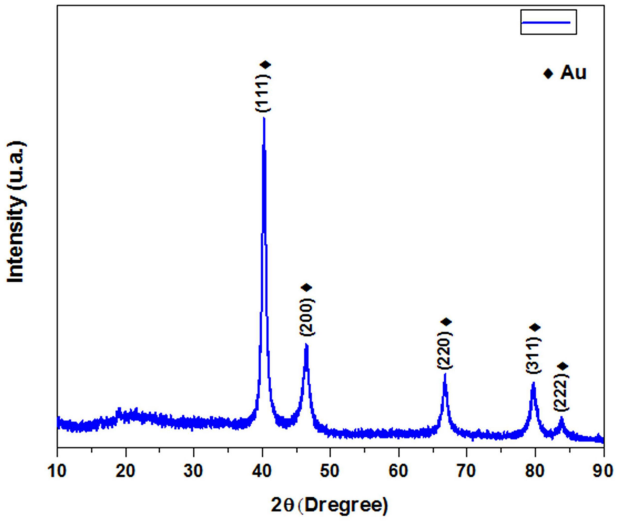

Fig. 10 *XRD pattern of the gold nanoparticles synthesised by H. brasiliensis latex*

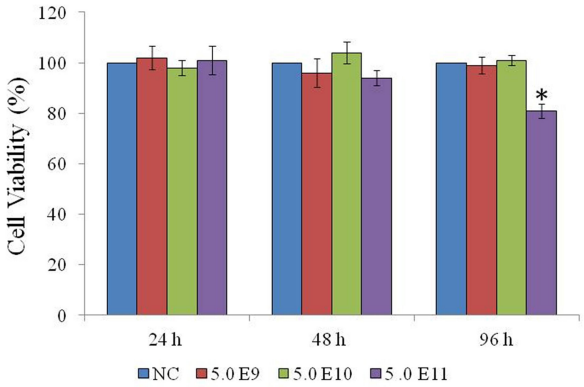

Fig. 11 *Cell viability (%) of CHO-K1 cells exposed to different concentrations of Au NPs or culture medium (NC) for 24, 48, and 96 h determined using the MTT assay. The horizontal lines represent the mean, and vertical lines represent the SD. *Indicates a significant difference compared with the respective NC ($p < 0.05$)*

The reduction in cell viability following exposure to a high concentration of NPs in our study could be due to increased apoptosis. Several in vitro studies found apoptosis induction by green synthesised gold nanoparticles [59, 60].

3.10 Genotoxic potential

The results of the cell viability test indicated that NPs at concentrations up to 5.0 E10 particles/mL were not cytotoxic towards CHO-K1 cells after a 96-h exposure. However, sublethal cellular changes may occur, which can alter certain cellular functions but do not result in cell death [61]. Here, DNA damage in CHO-K1 cells was evaluated by the comet assay following exposure to different concentrations of Au NPs synthesised using the latex of *H. brasiliensis*. The results indicated that, at the lowest concentration and shortest exposure time, the Au NPs synthesised were not genotoxic to CHO-K1 cells (Fig. 15). Danna *et al.* (2016) [18] found that silver NPs synthesised from natural rubber membrane prepared with *H. brasiliensis* latex presented low genotoxicity towards CHO-K1 cells after 24 h of exposure. An increase in genotoxic damage was observed with the highest concentration of Au NPs (5.0 E11 particles/mL) after 48 h of exposure, and with Au NPs at concentrations of 5.0 E10 and 5.0 E11 particles/mL after 96 h of exposure, indicating that Au NPs led to DNA damage in the exposed cells.

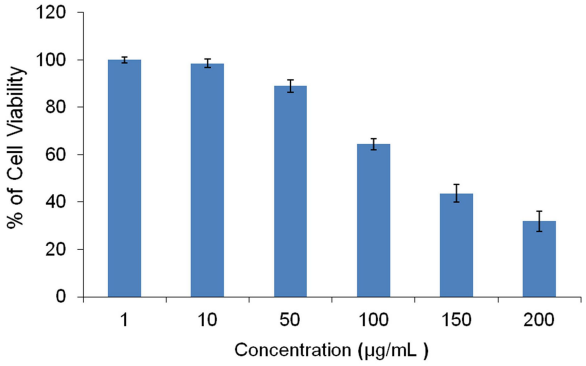

Fig. 12 *Cell viability (%) of CHO-K1 cells exposed to different concentrations of Au NPs or culture medium (NC) for 24 h. determined using the MTT assay, with the objective of calculating the LC50 The horizontal lines represent the mean, and vertical lines represent the SD*

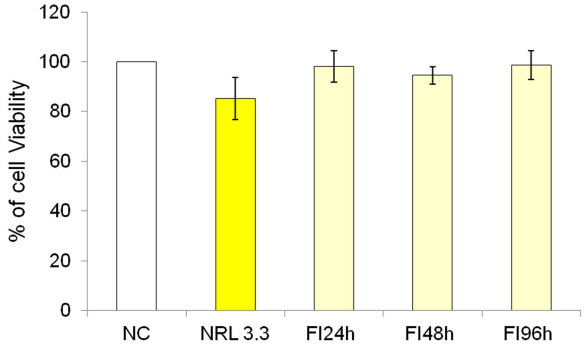

Fig. 13 *Cell viability (%) of CHO-K1 cells exposed to NRL 3.3 or culture medium (NC) for 24 h. Cell viability (%) of CHO - K1 cells exposed to FI at different exposure times also being shown*

4 Conclusion

Herein, the authors report the optimisation of a green synthesis method of Au NPs using an aqueous solution of *H. brasiliensis* latex as a reducing/stabilising agent. The optimal pH range for obtaining homogenous NPs with small sizes was 7.0–8.0. Temperature increased along with the velocity of the reaction without any influence on the formation of NPs.

A reduction time of 120 min allowed the maximum yield of Au NPs. The concentration of latex solution was fixed at 1.0 mmol/L, which was ideal for the green synthesis of Au NPs. From the toxicological results reported herein, the authors concluded that Au NPs at a concentration of 5.0 E9 particles/mL presented minimal cytotoxicity and genotoxicity, indicating that this concentration is biocompatible in normal cells. Although more studies are needed, Au NPs at this non-toxic concentration may be used in cell imaging and anticancer treatments. The identification of doses that do not cause toxicity in normal cells, but are toxic only to target cells, is a major challenge in medicine.

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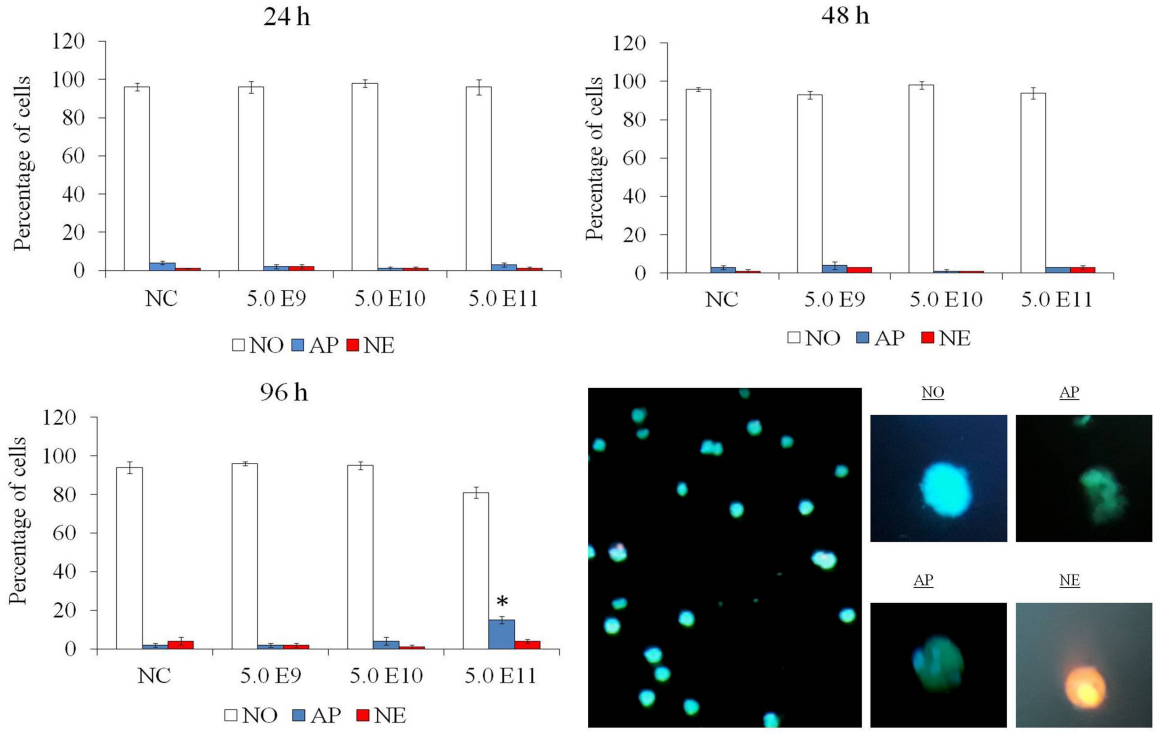

Fig. 14 *Percentage of normal cells (NO), apoptotic cells (AP), and necrotic cells (NE) among CHO-K1 cells exposed to different concentrations of AuNPs or culture medium (NC) for 24, 48, and 96 h. *Indicates a significant difference compared with the respective NC ($p \leq 0.05$). The large image shows NC cells at 96 h*

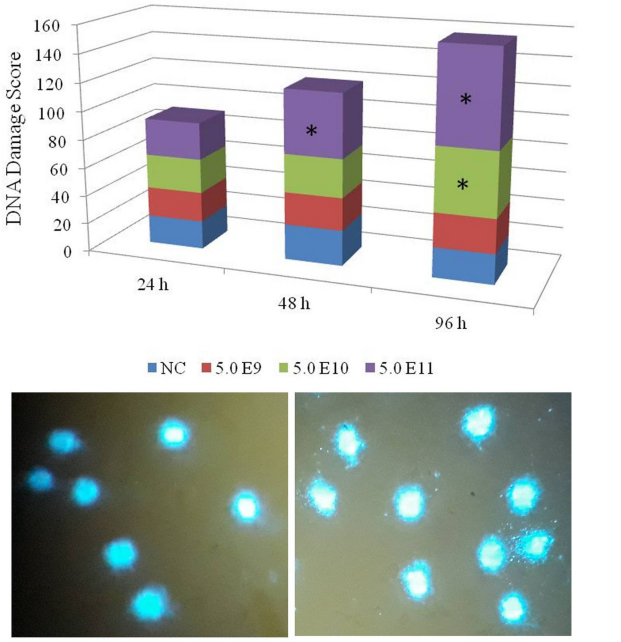

Fig. 15 *DNA damage scores in CHO-K1 cells exposed to different concentrations of Au NPs or culture medium (NC) for 24, 48, and 96 h were quantified by the comet assay. *Indicates a significant difference compared with the respective NC ($p \leq 0.05$). The picture on the left shows isolated nuclei of NC cells at 96 h; the picture on the right shows isolated nuclei of cells exposed to Au NPs at a concentration of 5.0 E11 at 96 h*

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