Antiviral and antioxidant properties of green synthesized gold nanoparticles using *Glaucium flavum* leaf extract

FatemehSadat Dehghani1 · Sareh Mosleh-Shirazi2 · Mostafa Shafiee1 · Seyed Reza Kasae3 · Ali Mohammad Amani1

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Abstract
Nowadays, nanoparticles such as gold nanoparticles (Au NPs) with specific biophysical characteristics have attracted remarkable attention as innovative options for the diagnosis and treatment of different diseases. In the present research, Au NPs were green synthesized using the *Glaucium flavum* leaf extract as an inexpensive and eco-friendly synthesis method. Then, the physicochemical properties were characterized by transmission electron microscopy (TEM), dynamic light scattering method (DLS), scanning electron microscopy (SEM), X-ray diffraction (XRD), Ultraviolet–visible absorption spectroscopy (UV–Vis), Zeta potential, and Fourier transform infrared (FTIR) spectroscopy. Afterwards, the antioxidant capacity was tested and antiviral activity against influenza virus was evaluated by applying TCID50 and PCR assays. The nanoparticles cytotoxicity was tested using the MTT method. The shape and size of Au nanoparticles were modulated by varying leaf concentrations with face-centered cubic (FCC) structure. At higher concentrations, long-time stable spherical nanoparticles were obtained with a mean particle size of 32 nm and low aggregation degree that could simply combine with various bioactive compounds. The outcomes exhibited effective antiviral and antioxidant activities with low cytotoxicity and acceptable biocompatibility of green synthesized Au NPs. The aim of the present study was to develop a potentially environmentally friendly nanoplatform with excellent antiviral and antioxidant functions and acceptable biocompatibility for promising biomedical applications in the future.

Keywords Green synthesis · Gold nanoparticles · Antioxidant · Antiviral · *Glaucium flavum*

Introduction
Nowadays, nanotechnology has offered innovative options for the treatment of different diseases. The anticancer, antioxidant, and antibacterial potentiality of nanoparticles has been widely explored (Chavan et al. 2021; Mosleh-Shirazi et al. 2021a, b). Among nanomaterials, gold nanoparticles (Au NPs) have attracted remarkable attention as a result of their potential biomedical applications, such as imaging and treatment of diseases (Mosleh-Shirazi et al. 2022). Au NPs are characteristically considered as bio-safe materials to apply as drug transport and antimicrobial materials (Selvaraj et al. 2010; Mosleh-Shirazi et al. 2021a, b; Atapour et al. 2022; Meisami et al. 2022).

Epidemic viral diseases, such as influenza and coronavirus, usually impose significant burden on the public health care system that necessitates novel antiviral therapeutics. Current antiviral drugs are only effective against some definite types of viruses; for example, there are limited antiviral drugs approved to treat the influenza virus (De Clercq and Li 2016). Recently, many investigations have focused on applying metal nanoparticles, especially gold and silver NPs, in combination with other natural bioactive agents for antiviral treatments (Galdiero et al. 2011; Meléndez-Villanueva et al. 2019).
However, the possible toxicity of Au NPs through oxidative stress or other mechanisms must be carefully evaluated before the clinical application (Tao 2018; Chen et al. 2020). Oxidative stress is considered as excessive free radical production, such as reactive oxygen species (ROS), over the antioxidant defense system. ROS not only can induce cytotoxicity for normal cells but also can interfere with the antiviral function of gold nanoparticles and could trigger many critical diseases such as Alzheimer’s, myocardial infarction, and cancers (Li et al. 2016; Lin et al. 2018).

In recent years, antioxidant properties of metal nanoparticles and their oxide derivatives have been studied in many researches (Liu et al. 2017; Desai et al. 2018; JadHAV et al. 2018; Lopez-Chaves et al. 2018). Moreover, green synthesized metal nanoparticles by applying various plant based phytochemicals such as terpenoids, flavonoids, and phenolic compounds, were also investigated to increase the antioxidant properties of these nanoparticles (Kajita et al. 2007; Vilas et al. 2016).

Furthermore, considering the biosensors and nanomedicine applications of Au NPs, it is necessary to produce nanoparticles with high stability in different environmental conditions such as high concentration ions and various temperature or pH conditions. For this purpose, many natural extracts or substances has applied for the biosynthesis of stable Au NPs (Zhang et al. 2021).

The green synthesis approach has gained much attention as an economical and environment-friendly technique that enables the synthesis of various shapes and size of nanoparticles with excellent stability and biocompatibility (Dehghani et al. 2022). Lee et al. performed some active ingredients extracted from plants, containing isoflavones (IF), protocatechuic acid (PCA), and Gallic acid (GA), play role as reducing agents to green synthesize functionalized Au NPs with excellent stability and biocompatibility that could be stable for three months. Since the high surface charge of the hydroxyl groups in the phytochemical composition, the strong repulsion between them could inhibit the agglomeration of Au NPs (Lee et al. 2011). Furthermore, the hydroxyl groups of compounds (as phenols, alkaloids, tannins, reducing sugars, flavonoids, and saponins) could reduce Au\(^{3+}\) to Au, and the plant extract’s carbohydrates could stabilize Au NPs (Singh et al. 2016; Ankamwar et al. 2017). Overall, HAuCl\(_4\) binds to plant extracts through chlorine–carbon bonds (Ghosh et al. 2011). In addition, starch and glucose components of the plant extracts could play role as stabilizers and reduce agents for synthesis of Au NPs with long-term storage and stability of 17 months (Engelbrekt et al. 2009).

One of the plants have been applied for green synthesis of metal nanoparticles is *Glaucium flavum* (GF), an annual plant native to Northern Africa, Europe, and Western Asia (Bercu et al. 2006). This plant is rich in alkaloid compounds such as aporphine, protoberberine, and protopine, among which (Glaucine) is the most important alkaloid. Research has shown the antimicrobial function of this plant that some medications derived from this plant extract applied as adjuvant treatment in respiratory disorders or antibiotic (Zhang et al. 2008). The present study describes a simple method to synthesize Au NPs by applying *Glaucium flavum* extracted as the eco-friendly and low-cost stabilizing and reducing. In addition, the physical characteristics, antioxidant properties, and antiviral activity against the H1N1 virus were investigated in order to ameliorate gold nanoparticles in biomedical applications as antioxidant delivery vehicles with antiviral properties.

### Materials and methods

The *Glaucium flavum* (yellow horned poppy) plant from the subfamily of *Papaveraceae* has gathered from Kam-firuz city in Fars province. Initially, the plant was washed with distilled water to eliminate the pollution, parched, and powdered at ambient temperature. The combination of 5 g powder and 100 ml distilled water was heated for 15 min. Subsequently, cooling down the solution for 24 h, filtered through Whatman filter paper, and centrifuged three times to obtain a uniform solution. This fresh extract was preferably refrigerated. 1 ml of this product and 1 ml of 10 mM HAuCl\(_4\) (99.0%, Merck Company) were stirred for 1 h to obtain colloid g1. The addition of the extract was varied to 3, 5, 7, and 9 ml to obtain colloid g2 to g5, respectively.

The morphology and size of the green synthesized GF-Au NPs were evaluated by a Philips CM 10 TEM microscope. TEM sample was also prepared with a few drops of well-scattered NPs on a 300-mesh grid of C-coated copper, then dried at ambient temperature. Dynamic light scattering method (DLS) was applied by a MALVERN Zen3600 to investigate the size distribution of NPs. X-ray diffraction (XRD) was performed to evaluate the crystallinity and composition of the NPs by a Siemens D5000 X-ray diffractometer with Cu Kα radiation. Ultraviolet–visible absorption (UV–vis) was applied by a Varian Cary 50 UV vis-spectrophotometer between 400 and 800 nm. Fourier transform infrared (FTIR) was applied to investigate the functional groups of GF-Au NPs by a Bruker VERTEX 80 v and the KBr disk method.

### Antioxidant activity protocol

Methanol solution of DPPH discoloration test was performed to evaluate the radical scavenging capability of (GF-Au NPs) and particularly, this discoloration technique was operated by DPPH reduction. The DPPH solution interacts with the different concentrations of GF-Au NPs (from 125
to 1000 µg/ml as a reductant agent that the DPPH color changed from violet to yellow. After vigorous stirring for 30 min of the solution, the microplate reader at 517 nm was applied to determine the exact color change. Butylated hydroxytoluene (BHT) was considered as the positive control agent and DPPH radical scavenging activity, was considered as the inhibition percentage (Rabiee et al. 2020). This analysis was completed within three times, and according to the concentration of the sample that needed to scavenge half of the DPPH free radical (IC50), the inhibition curves were drawn.

**Antiviral protocol**

The Madin–Darby canine kidney (MDCK)-SIAT1 cells (obtained from the Razi Vaccine and Serum Research Institute, Karaj, Iran) were grown in 5% CO2 in Dulbecco’s Modified Eagle Medium (DMEM) at the temperature of 37 °C, supplemented with heat-inactivated fetal bovine serum 10% (FBS), 100 µg/ml streptomycin sulfate, 100 U/ml penicillin, 1 mM sodium pyruvate (Merck Germany), 100 µg/ml streptomycin sulfate (Sigma-Aldrich, USA), and 2 mM L-glutamine. For the preparation of virus-stock, the monolayer of MDCK-SIAT1 cell in the 25-cm² flask (SPL Life Science) was deterged with phosphate-buffered saline three times, and the obtained cells were infected with the Influenza A/ Puerto Rico/8/34 (H1N1; PR8) virus obtained from the Razi Vaccine and Serum Research Institute at a multiplicity of infection (MOI) for 1 h at 35 °C. After removal of the virus inoculum, covered cells with the infection medium with serum-free DMEM, 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (Sigma-Aldrich, USA) buffer, 0.14% of bovine serum albumin (Sigma-Aldrich, USA), and 2 µg/ml trypsin-TPCK (Merck, Germany). Then, the flask was incubated for 48 h at 35 °C. After 48 h post-infection, the virus-containing supernatants were harvested and centrifuged at 3000 rpm for 10 min, eventually filtered through a sterile syringe filter 0.22 µm. Then, the virus was frozen at –80 °C prior to use. The virus was titrated through the 50% tissue culture infectious dose (TCID50) according to Reed and Muench formula method (LaBarre and Lowy 2001) and was used for the next in vitro experiments at the titer of 100 TCID50/ml.

To assess the effects of GF-Au NPs nanoparticles on the H1N1 influenza virus, equal volumes of the viral suspensions (100 TCID50/ml) were mixed with different concentrations of nanoparticles (from 10 to 250 µg/ml) and incubated at 37 °C for 4 h. The mixture (100 µl) was then added in triplicated wells of the confluent monolayer of MDCK-SIAT1 cells (2 × 10⁴ cells/well) in 96-well microplate and further incubated for 1 h at 35 °C. The untreated infected tubes were considered as negative control group. After 1 h incubation, the mixture was removed and the cells were washed three times with PBS to remove non-absorbed viruses and overlaid with infection medium. The plate was then incubated for 48 h at 35 °C and 5% CO₂ condition. This assay protocol was also performed for oseltamivir. After 48 h, the supernatant of each well was collected and subjected to TCID50 and quantitative real-time PCR assays to determine the amount of viral load.

**MTT assay protocol**

Cytotoxicity research was operated using a dermal Fibroblast cell line obtained from the Pasteur Institute’s National Cell Bank of Iran. The tissue fibroblast cells were cultured in the 96-well plate with the 10⁵ cells per well standard density for 24 h at standard conditions (37 °C, 5% CO₂ in incubator). Subsequently, the culture media of 10% fetal bovine serum (FBS) were removed, and the cells were washed two times with phosphate-buffered saline (PBS). New maintenance Roswell Park Memorial Institute (RPMI) medium (10% FBS) was replaced with various concentrations of GF-Au NPs (50, 100, 150, 200, and 300 µg/ml) and incubated for a further 72 h. Tube cultured cells that did not treat with GF-Au NPs were mentioned as negative controls. A 10 µl solution of freshly prepared 5 mg/ml MTT in PBS was well added to each and incubated for an additional 4 h. The media was removed, and 100 µl of Dimethyl sulfoxide (DMSO) was used to dissolve the generated 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) formazan. Each well’s absorbance was evaluated using the microplate reader at 570 nm. Cell toxicity and viability percentages were calculated by subsequent formula:

\[
\text{Toxicity\%} = \left(1 - \frac{\text{MeanODofsample}}{\text{MeanODofcontrol}}\right) \times 100
\]

\[
\text{Viability\%} = 100 - \text{Toxicity\%}
\]

The IC50 (50% inhibition of cell growth) values of GF-Au NPs were calculated following 24, 48, and 72 h of exposure times. The experiment was repeated three times and applied for the measurements.

Statistical Package for Social Sciences (SPSS) software (version 20.0) was performed to statistically analyze data. Data were determined as mean ± standard deviation (SD). Statistical significance was determined as a P value less than 0.05. To compare MTT data between two groups and different groups, the Student’s t test and Analysis of Variance (ANOVA) followed by the Tukey post hoc test were applied, respectively.
Results and Discussion

UV–Vis analysis

The UV–Vis absorption spectra of processing Au NPs with different leaf concentrations (g1–g5) are shown in Fig. 1. The SPR absorbance is greatly sensitive to the shape, size, and nature of the particles and the surrounding medium (Mosleh-Shirazi et al. 2021a, b). At lower leaf concentration (g1), the Surface plasmon resonance (SPR) peak is broad with long wavelength absorption that indicates considerable anisotropy in the shape of Au NPs. As the leaf concentration increases, the band becomes a blue shift towards a lower wavelength from g1 to g4 (Fig. 1). Eventually, in colloid (g5), a sharper absorption peak at 541 nm is detected as a characteristic of spherical particles (Kasthuri et al. 2009; Philip 2009).

Morphology analysis

TEM images of Au NPs are displayed in Fig. 2 and indicate that the shape and size of the NPs are sensitive to leaf concentration. The morphology of Au NPs consists of a mixture of hexagonal, spherical, and triangular like particles with a decline in aptitude and size of triangular particles with increment in leaf concentration (Figure S1 and Figure S2). The morphology of colloid g5 (Fig. 2e) revealed that most spherical shape NPs had a low aggregation degree with a mean particle size of 32 nm. As the amount of biomolecules that act as coupling agents increases, the formation of spherical particles increases relative to the triangle. The rest of the analyses were performed on colloid g5.

Figure 3 represents the particle size distribution diagram for Au NPs gained from the DLS technique, indicating that Au NPs had the average size of 51.4 nm (Fig. 3a). These results are not consistent with the results of the TEM. Nevertheless, the differences in nanoparticles’ average size between DLS and TEM techniques have been determined in previous research (Singhal et al. 2011; Kouhbanani et al. 2019; Lohrasbi et al. 2019). The stability of the size distribution of Au NPs was evaluated after 2 years (Fig. 3b), indicating that the size of gold nanoparticles reached 112.8 nm. Recently, excluding demanding the significant stability of Au NPs during synthesis, the final stability of nanoparticles should be considered that is very essential for the storage and application of Au NPs, such as cancer therapy and bioimaging (Zhang et al. 2021; Sangwan and Seth 2022).

The zeta potential is the surface charge potential index as well as an important parameter to determine the stability of nanoparticles in the suspension (Erdogan et al. 2019). The zeta potential value of immediately and 2 years after synthesis of Au NPs were −83.3 and −82.0 mV, respectively. Research studies have revealed that the zeta potential for gold nanoparticles is −42 and −62 mV, respectively (Majzik et al. 2009; Raj et al. 2011), which indicates green synthesized GF-Au NPs are more stable. The negative value of the zeta potential of the Au NPs indicates their long-term and significant stability in the suspension. Furthermore, the GF-Au NPs had warped with anionic compounds, and thus interparticle electrostatic repulsive force lead to preclude aggregation of the nanoparticles. Higher the negative value of Zeta potential reveals more interparticle repulsion and stability.

XRD pattern

X-ray diffraction patterns (XRD) of the Au NPs indicates in Fig. 5. The diffraction peaks at 2 theta = 38.2°, 44.5°, 64.7°, and 77.6° were indexed as the (111), (200), (220), and (311) planes of FCC structured of Au NPs, respectively. Furthermore, no significant peak was observed in the sample that had been synthesized for 2 years, indicating that the structure was amorphous (Fig. 5b). No contamination or impurities were detected. Furthermore, the broad peak of about 10° indicates the nanoparticle’s surface is covered with organic
biomolecules (Beheshtkhoo et al. 2018). Amorphous and crystalline materials composed of the same atoms reveal significant different properties. Furthermore, the behavior of materials’ composed of mesoscale particles is strongly related to their component particles’ arrangement. Many particle simulations indicate that the contact mechanics of the ligand shells overcome the disorder-order transition. Hence, the interactions of purely spherical particle–particle lead to order and crystalline structure; in contrast localized stiction between the ligand shells of Au NPs leads to amorphous and disorder conversions (Geyer et al. 2012). This reveals that the packing of the agglomerates and the stickiness could be changed by the ligand shells’ state. These switching between crystallization and amorphous agglomeration of functionalized Au NPs were previously reported by other researchers (Compton and Osterloh 2007; Geyer et al. 2012).

**FTIR analysis**

Figure 6 shows the FTIR analysis performed to evaluate the substantial biomolecules that play a role in capping and stabilizing green synthesized GF-Au NPs. Intense absorptions are detected at 3439, 2076, 1637, and 634 cm$^{-1}$. The IR band at 3439 cm$^{-1}$ refers to the OH stretching mode of the carbohydrates proteins and polyphenols. The band detected at 2076 and 634 cm$^{-1}$ was associated with the C≡C stretching of the alkynes group and C–N/C–Cl in plane bending, respectively. Furthermore, the IR band at 1637 cm$^{-1}$ is characteristic of the C=O stretching of the carboxylic group. Therefore, it is probable that enzymes/proteins play a role in the reduction of metal ions by the oxidation of aldehydes to carboxylic acid. Proteins can attach to Au NPs through carboxylate ions of amino acid residue or free amine groups in
it (Smitha et al. 2009; Philip 2010). Furthermore, the existence of the C=O stretching mode reveals the existence of the –COOH group in the material bound to Au NPs. Therefore, the band at 1637 cm\(^{-1}\) represented the probability of proteins binding on Au NPs through free amine groups.

**Antioxidant analysis**

Oxidative stress can release free radicals, such as ROS, interacting with cytoplasmic enzymes or DNA that aggravate or...
propagate complications of some critical diseases such as metastatic cancer, diabetes retinopathy, or neuropathy. In addition, during biological functions of nanoparticles, ROS products may release that can interfere with TNF receptors or pro-inflammatory cytokines (IL-6 and TNF-α) and decrease the immune function or induce cytotoxicity. Previous studies also revealed that preventing ROS accumulation is an important factor affecting the antiviral function of metal NPs against the different types of viruses (Li et al. 2016; Lin et al. 2018). Therefore, it is necessary to evaluate the free radical production or transporting capacity of Au NPs while evaluating the antiviral functions (Elsabahy and Wooley 2013).

Hence, the concomitant antioxidant and antiviral properties of green synthesized NPs will be potentially valuable issues. In the present research, the ability of Au NPs to scavenge free radicals was investigated by the NPs’ capacity to trap the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. The GF-Au NPs revealed a good potency in a dose-dependent pattern to trap DPPH free radicals. The 23% DPPH reducing ability was achieved at the concentration of 125 μg/ml and then reached 37% and 44% at 500 and 1000 μg/ml of GF-Au NPs, respectively (Fig. 7).

The probable mechanism for the antioxidant properties of the GF-Au NPs could be related to some *Glaucium flavum* phytochemical components such as phenolic, alkaloids, and flavonoid compounds, which was confirmed by phytochemical analysis and FTIR results. Through the green synthesis of the Au NPs, these bio-compounds could attach to the Au NPs surface to increase the antioxidant function and different concentrations of antioxidant extracts could produce concomitantly.

Previous studies have confirmed that Au NPs composed or stabilized with natural antioxidants reveal better antioxidant activities due to the free radicals scavenging capacity of these biometabolites (Singh et al. 2013; Shabestarian et al. 2016).

In consistent with other researches, the results of FTIR analysis detected the better surface area of the green synthesized GF-Au NPs in comparison with traditional Au NPs (Jayaseelan et al. 2013; Begum et al. 2022). It could be explained that through the green synthesis of the Au NPs, the bioactive components attached on Au NPs surface increasing surface area to volume ratio, with high propensity to interact with free radicals and reduce DPPH involve in the antioxidant properties. Thus, the bioactive-derived components of *Glaucium flavum* plant contributed in the green synthesis of Au NPs had led to the improvement free radical scavenging activity of Au NPs (Veeramani et al. 2022).

In addition, the synthetic method, the type of spin–spin metal relationships, or morphology, which depend on several factors, could influence the antioxidant properties. For example, if the synthetic method uses more aldehyde or phenolic compounds, the antioxidant property could be increased substantially.

### Antiviral analysis

In the present study, we investigate the ability of GF-Au NPs as an antiviral alternative for therapeutic procedures against *H1N1* by TCID50 and PCR assays, and the results were compared with oseltamivir, one of the most effective antiviral drugs against *H1N1* (Table 1).

The outcomes revealed that 50% inhibition of viral replication was achieved at the concentration of 210 μg/ml Au NPs, and antiviral activity of 60% was gained with the concentration of 250 μg/ml.

Green synthesized Au NPs are significantly stable (Fig. 3) and could facile interact with various bioactive components of the *Glaucium flavum* extract, such as alkynes, protein-based compounds, and phenolic compounds (Fig. 6).

Some previous research has revealed that bioactive metabolites contributed to the green synthesis of nanoparticles, such as alkaloids and phenols, not only increase the stability of nanoparticles but also can improve the antiviral

### Table 1 In vitro antiviral activity of oseltamivir and Au NPs against influenza virus (*H1N1*) using the tissue culture infectious technique

| Inhibition rate (%) | Concentration (μg/ml) |
|---------------------|-----------------------|
|                     | 10  | 20  | 50  | 80  | 100 | 150 | 200 | 250 |
| Oseltamivir         | 12  | 58  | 93  | 95  | 97  | 97  | 98  | 98  |
| Au NPs              | 3   | 18  | 28  | 54  | 38  | 39  | 46  | 60  |
characteristics of gold or silver nanoparticles (Haggag et al. 2019; Meléndez-Villanueva et al. 2019).

The antiviral function of metal NPs can occur either through intracellular or extracellular mechanisms. For example, by blocking the binding site of gp120 protein on the cell membrane, Au NPs can interrupt viral attachment or penetration before invading host cells. Au NPs also can bind to the viral genome and disable the virus particles before cellular entry. Furthermore, the inhibition of intracellular viral replication enzymes is another antiviral capacity of Au NPs that inhibit viral duplication (Galdiero et al. 2011; Rai et al. 2016; Aderibigbe 2017; Maduray and Parboosing 2021).

Nanoparticle size, shape, and surface charge are important factors that play a role in cellular uptake and antiviral function (Foroozandeh and Aziz 2018; San and Shon 2018). Positive charged NPs such as gold nanoparticles efficiently endocytosis and accumulate in the cytoplasm through negative charge cell membrane receptors (Jiang et al. 2008; Foroozandeh and Aziz 2018). The results of the present study revealed that polyphenols of plant extract act as a covering agent to inhibit particle aggregation and induce better NPs distribution. It is suggested that the biometabolites of Glaucium flavum plant (flavonoids and phenols) can increase the gold nanoparticles’ antiviral function against the H1N1 virus by enhancing its ability to interact with the viral genome or binding site at the host cell membrane. In addition, modulating the surface charge can enhance cell entry and inhibit the specific viral replicative enzymes in the cytoplasm.

The results of the study revealed the effective antiviral function of Au NPs Glaucium flavum extracted against the H1N1 virus obtained from the antiviral characteristics of both Glaucium flavum biometabolites and Au NPs.

Previous researches have detected that some plant base biocomponents have essential antiviral properties, which in combination with gold nanoparticles, can increase the antiviral properties of metal nanoparticles (Fatima et al. 2016).

In addition, gold nanoparticles can interact with immunocytes (macrophages, or lymphocytes) and potentially stimulate host immune responses against viral infection (Engin and Hayes 2018).

Related to the immunomodulatory or antiviral properties of Au NPs, it could be designed as the vaccine or adjuvants antiviral agent in the future.

However, cytotoxicity and biocompatibility of nanomaterial should be considered before any biomedical application (Dobrovolskaia and McNeil 2007; Look et al. 2010; Pandey and Prajapati 2018).

**MTT assay**

To assess the cytotoxicity of the green synthesized GF-Au NPs, cells were treated with varying concentrations from 24 to 72 h (Fig. 8). The results indicated that after 24 h, there is the minimal decrease in cell viability in contact with Au NPs of 50 to 200 μg/ml with no significant difference between these concentrations at the first 24 h (P value > 0.05); however, by increasing the concentration to 200 μg/ml the cytotoxicity and cell death significantly increases after 48 h. Indeed, the prominent cytotoxicity became apparent after 72 h that the MTT assays revealed significant cytotoxicity for Au NPs of concentration ≥ 150 μg/ml. The biosynthesized Au NPs have no biological cytotoxicity at concentrations of 50 and 100 μg/ml, even after 72 h. According to advanced biomedical applications of gold nanoparticles, the cytotoxicity of Au NPs is an important point in biotechnology research. There are still contrary outcomes from different studies evaluating the cytotoxicity of gold nanoparticles.

A great number of in vitro and in vivo experiments have confirmed the nontoxicity and biosafety of Au NPs, while some evidence indicates the toxicity of Au NPs for vital cells (Singh et al. 2013). This could be due to different standard bases of the toxicity studies that explain the contrary results (Minetto et al. 2017). Indeed several aspects, such as shape, size, surface charge, NPs concentration, and exposure time, could affect gold nanoparticles’ cytotoxicity (San et al. 2021; Mosleh-Shirazi et al. 2021a, b). However, the type of cells selected for the cytotoxicity test is a conflicting issue in biosafety research because some cells, such as macrophages, fibroblasts, or epithelial cells, have revealed more sensitivity to gold particles (Pan et al. 2007).

It has been shown that Au NPs less than 30 nm efficiently endocytosis through the cell membrane and induce cell apoptosis by different mechanisms, such as DNA damage or interaction with cytoplasm enzymes (Haggag et al. 2019). In contrast, greater sized nanoparticles cannot easily enter cells and show low toxicity or adverse effects for normal cells.
The MTT assay analysis confirmed the low toxicity of green synthesized GF-Au NPs for fibroblast cells. The results of TEM images revealed the spherical shape and average size of 32 nm for GF-Au NPs that could interrupt with endocytosis of these NPs by normal cells and significantly decrease the related cytotoxicity. In addition, the low cytotoxicity results of the biosynthesized Au NPs may be attributed to their functionalization with organic moieties derived from the *Glaucium flavum* extract.

Some other studies have also detected that gold NPs functionalized and stabilized, with different bioactive components exhibit better biodistribution and less cytotoxicity due to the enhanced bioactivity of these molecules (Tiwari et al. 2011; Al Saqr et al. 2021).

In addition, further studies should be conducted through the cytotoxicity of GF-Au NPs on laboratory animals and in vivo conditions to support the nanoparticles’ biocompatibility. In addition, it is critical to consider the ecotoxicity of Au NPs to develop novel insights into ecotoxicological conflicts (Libralato et al. 2017).

**Conclusions**

There is substantial interest in the investigation of biomedical applications of Au NPs. In the present study, *Glaucium flavum* extracts were used to synthesize various shapes and sizes of Au NPs. Various characterization methods such as UV–Vis, Zeta potential, SEM, XRD, DLS, TEM, and FTIR have supported the successful synthesis of highly stable Au NPs. The shape and size of Au nanoparticles are modulated by varying leaf concentrations with FCC structure. At higher concentrations, long-time stable spherical nanoparticles were obtained with a mean particle size of 32 nm and a low aggregation degree that could simply combine with various bioactive compounds. The zeta potential value of immediately and 2 years after the synthesis of Au NPs were −83.3 and −82.0 mV, respectively. The negative value of the zeta potential of the Au NPs indicates their long term and significant stability in the suspension. Furthermore, Au NPs could simply combine with various bioactive compounds conforming to the *Glaucium flavum* extract (alkynes, protein-based, and phenolic compounds), which would confer Au NPs characteristics to improve their biological activity. Green synthesized GF-Au NPs exhibit efficient antioxidant and antiviral functions with acceptable safety that could be mentioned as an antioxidant agent with related applications to treat or prevent cancers or adjuvant treatment against the influenza H1N1 virus in combination with other proven treatments. However, there are still conflicting subjects concerning the biomedical applications of Au NPs that further studies should be established to evaluate the Au NPs’ properties to determine these challenges.

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**Declarations**

**Conflict of interest** The authors declare that there is no conflict of interest regarding the publication of this article.

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