Cancer Nanotechnology

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Using green biosynthesized kaempferol-coated sliver nanoparticles to inhibit cancer cells growth: an in vitro study using hepatocellular carcinoma (HepG2)

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Abstract

The ongoing loss of human life owing to various forms of cancer necessitates the development of a more effective/honorable therapeutic approach. Moreover, finding a novel green-synthesized anti-cancer therapy is vital because of the induced drug resistance against the commonly used drugs. Collecting the advantage of the nanometer size of nanoparticles with the biosafety of plant-based substances might potentiate the anticancer effect with minimal toxic effect. In the current study, we aimed to green-synthesize using kaempferol (flavonoid) as a coating the silver nanoparticles (AgNPs) and investigated their anti-cancer activity in hepatocellular carcinoma (HepG2) cell line. First of all, kaempferol-coated AgNPs characters were well-defined using Fourier transmission infrared (FTIR), X-ray diffraction (XRD), zetasizer, and transmission electron microscopy (TEM). The results showed their 200 nm size, spherical shape, less aggregation with high stability characteristics. Then, the cytotoxic effect of both 1/3 and 1/2 LC50 of AgNPs, and doxorubicin (DOX, anticancer drug) on HepG2 cells was evaluated by dimethylthiazolyltetrazolium bromide (MTT) assay and release of lactate dehydrogenase (LDH) leakage percent. Reactive oxygen species (ROS) and apoptotic markers were also analyzed, along with the migration and invasion of HepG2 cells were recorded. Our findings showed that kaempferol-coated AgNPs could induce cytotoxic effects and reduce the viability of HepG2 cells in a concentration-dependent manner. LDH leakage % was significantly increased in cells treated with kaempferol-coated AgNPs confirming their cytotoxic effect. ROS generation and lipid peroxidation could significantly increase in HepG2 cells treated with kaempferol-coated AgNPs along with the exhaustion of antioxidant Glutathione (GSH) marker revealing the induced oxidative damage. Oxidative damage-mediated apoptosis was confirmed by the elevated levels of the pro-apoptotic markers (Bax, Cyt-c, P53, and caspase-3) and the reduced level of anti-apoptotic marker (Bcl-2) using enzyme-linked immunosorbent assay (ELISA). Furthermore, kaempferol-coated AgNPs could suppress the migrating and invading ability of HepG2 cells showing their antimetastatic effect. To end up, kaempferol-coated AgNPs can induce a potential anti-cancer effect in HepG2 cells via oxidative stress-mediated apoptosis.

Keywords: Silver nanoparticles (AgNPs), Kaempferol, HepG2 cells, Apoptosis, Oxidative stress

Introduction

Nanoparticles are widely used nowadays in the biomedicine field because of their unique chemical and physical properties related to their nanometer size (Menon et al. 2017; Murray et al. 2000; Othman et al. 2021b). Many different metal nanoparticles are employed in drug delivery, fluorescent biological labels, and the detection of pathogens and proteins (Menon and Shanmugam 2020; Wang et al. 2002). Silver nanoparticles (AgNPs) showed many biological activities, such as anti-inflammatory, anti-bacterial, and antifungal effects (Fehaid et al. 2020). Therefore, a biological-based substance should coat the nanoparticles to make them biocompatible, such as collagen (Sinani et al. 2003) and apigenin to improve the cellular–nanoparticles interaction (Al-Otaibi et al. 2022). Exposure to AgNPs causes oxidative stress associated with DNA damage, apoptosis, and necrosis. Hence, AgNPs have been shown to cause cytotoxicity in a variety of cancer cell lines, including prostate cancer cells (Firdhouse and Lalitha 2013), human Chang liver cells and rat basophil leukaemia (RBL) cells (Piao et al. 2011), lung carcinoma A549 cells (Gurunathan et al. 2015), MCF-7, and HCT-116 cells (Khan et al. 2021). AgNPs have also been shown to be more harmful to cancer cells than to non-cancerous cells (Faedmaleki et al. 2014).

Cancer is a leading cause of death worldwide, and liver cancer is one of the most common cancer types, which induced 830 000 deaths in 2020 as reported by the World Health Organization (WHO) (Adegbola and Ajayi 2008). Hepatocellular carcinoma (HCC) is the most common primary liver malignancy leading to liver cirrhosis and high mortalities percent (Balogh et al. 2016). Therefore, finding new strategies using nanotechnology to treat hepatic cancer is essential, particularly to plant-based nanomaterials therapy.

Flavonoids are distributed widely in many plants and were reported to inhibit tumor growth by initiating oxidative stress (De Stefani et al. 1999). Kaempferol (3, 5, 7, 4′ tetrahydroxy flavone) is one of the flavonoids present in different vegetables, such as broccoli and onions (Al-Brakati et al. 2021; Tatsimo et al. 2012). It was reported to treat many diseases because of its antioxidant, anti-inflammatory, and antitumor effects (Kim et al. 2016; Tang et al. 2015). In addition, kaempferol was used to synthesize gold nanoparticles successfully and could produce anti-cancer activity in breast cancer cells via an angiogenesis mechanism (Raghavan et al. 2015).

Owing to the reported different biological activities of both AgNPs (nano-based) and kaempferol (bio-based) separately, this study hypothesized that the biosynthesized AgNPs using kaempferol might show a synergistic effect of both nano- and bio-based systems against the growth of cancer cells. Therefore, the current *in-vitro* study aimed to investigate the potential anti-cancer effect of kaempferol-coated AgNPs in a hepatocellular carcinoma cell line (HepG2).

Materials and methods

Chemicals and reagents

Kaempferol, silver nitrate, dimethyl sulfoxide (DMSO), Dimethylthiazolyltetrazolium bromide (MTT), and 2,7-dichlorofluorescein diacetate (DCFH-DA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). RPMI-1640 medium was obtained from Gibco Life

Technologies. Heat-inactivated fetal bovine serum (HFBS) was procured from Invitrogen Co., (Waltham, MA, USA). Lactate dehydrogenase (LDH) CytoTox Non-radioactive cytotoxicity assay kit was supplied by Promega (Madison, Wisconsin, USA). ELISA kits for B-cell lymphoma 2 (Bcl-2), Bcl-2-like protein 4 (Bax), cytochrome-c (Cyt-c), tumor suppressor protein (P53), and Caspase-3 (Cas-3) and annexin V-FITC were obtained from Abcam (Cambridge, UK). Dulbecco's modified eagle's medium (DMEM) diluted Matrigel was purchased from BD Biosciences (USA). All other chemicals used were of the highest purity grade available from different commercial sources. Milli-Q water was used as a solvent for silver. DMSO was used to prepare stock solutions of kaempferol at a concentration of 1 mg/mL and stored in -20 °C for further use.

Cell culture

Hepatocellular carcinoma cell line (HepG2) was obtained from ATCC. HepG2 cells were cultured at 37 °C in 5% CO2 incubator in RPMI-1640 medium supplemented with 1% (v/v) penicillin–streptomycin and 10% (v/v) HFBS. When the confluency of cells reached 80%, trypsinization was used to detach and subculture cells. HepG2 cells were seeded at a density of 15,000 cells per well in 96-well plates for the treatments.

Kaempferol-coated AgNPs synthesis

A total volume of 10 ml of kaempferol (stock solution) and 90 ml of 0.1 M $AgNO_3$ solution was incubated together for 24 h at room temperature, turning the solution into the dark brown color of AgNPs. Then, the solution was heated and stirred at 90 °C for 15 min and centrifuged later at 9000 rpm at room temperature.

Kaempferol-coated AgNPs characterization

Fourier transmission infrared (FTIR) and UV–VIS spectral analysis were used to detect the optical density of kaempferol-coated AgNPs. X-ray diffraction (XRD) was used to know about the crystalline structure of AgNPs. Zetasizer (Nano series, ZEN 3600, Malvern, U.K.) was used to determine the average size. In addition, transmission electron microscopy (TEM) was used to examine morphology and aggregation.

Cells viability/cytotoxicity assay

MTT colorimetric assay was applied to detect the cytotoxicity of kaempferol-coated AgNPs. Briefly, HepG2 cells were incubated after seeding in 96-well plates with different concentrations of kaempferol-coated AgNPs and doxorubicin (DOX) for 24 h at 37 °C in a humidified incubator. Cells were then washed with 1× phosphate buffer saline (PBS) and treated with 20 µl of MTT (5 mg/ml) at 37 °C for 30 min. The formed crystals of formazan were dissolved using 200 µl of DMSO and re-incubated at 37 °C for another 30 min. The differences in color intensities were recorded at 570 nm using a microplate reader (Othman et al. 2021a).

LDH leakage assay was used to assess the cytotoxic effect of kaempferol-coated AgNPs. For this purpose, a colorimetric assay was used according to the manufacturer's instructions. Released LDH by cell lysis is measured in the supernatants with an enzymatic assay by converting the tetrazolium salt into red formazan. The intensity of the formed color is proportional to the number of lysed cells. The color intensities

(absorbance) differences were recorded at 490 nm using a microplate reader. In addition, the relative LDH leakage (%) was calculated and recorded related to the control cells (Braydich-Stolle et al. 2005).

Determination of apoptotic markers

ELISA kits were employed to analyze the protein levels of apoptotic biomarkers. Pro-apoptotic markers including Bax, Cyt-c, P53, and Cas-3 were measured along with the anti-apoptotic marker Bcl-2. As follow, 2×10^6 HepG2 cells were treated with kaempferol-coated or AgNPs (concentrations of 1/2 LC50 and 1/3 LC50). Furthermore, DOX at LC50 (11.02 µm/ml) as a positive control and cells with the vehicle as negative. After 24 h of incubation, cells were collected by centrifugation at 1800×*g* for 5 min to remove the medium and washed twice with PBS. The pellets were then lysed by pipetting in 50 µl cold lysis buffer. Next, the cell lysate was centrifuged at 12,000×g for 1 min at 4 °C, and the supernatant was collected. Protein levels of apoptotic markers were analyzed using the Bradford method in each lysate. Cell extraction buffer PTR was used for dilution of lysates if the level of protein exceeds 4 g/l. In the end, the resultant color of the samples was recorded at 405 nm in a microplate reader (Biotech, Inc., USA).

Migration and invasion assay

Corning Transwell 8.0-µm pore membranes (Corning, USA) were used to evaluate cell migration and invasion. Briefly, 1×10^5 HepG2 cells were seeded onto the upper chamber of each transwell. For invasion evaluation, the upper chamber of transwell was coated with 100 µL of 1:8 DMEM-diluted Matrigel before the seeding of HepG2 cells. Lower chambers of transwell were contained fresh medium as a chemo-attractant with the presence or absence of kaempferol-coated AgNPs or DOX. After incubation for 48 h, the cells remaining at the upper surface of the membrane were removed using a cotton swab, whereas those cells that had migrated or invaded to the lower membrane surface were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet solution. The number of HepG2 cells migrated and invaded through the filter was photographed and counted using a light microscope at a magnification of 200x.

Flow cytometry

HepG2 cells at a 2×10^5 cell/ml concentration were cultured in T_{25} cell culture flasks and treated with kaempferol-coated AgNPs or DOX. After 24 h, cells were colected and resuspended in a binding buffer. Then, annexin V-FITC (Cat. No: ab14085) was added and incubated at 30 °C for 10 min in the dark. Cell cycle distribution was analyzed by flow cytometry.

Oxidative stress determination

HepG2 cells at a 2×10^5 cell/ml concentration were cultured in T25 cell culture flasks and treated with kaempferol-coated AgNPs or DOX. After 24 h, cells were harvested and lysed in a lysis buffer. The lysates were then spun down by centrifugation at 12,000×*g* at 4 °C for 1 min, and the supernatant was collected. The Bradford method was used to determine the protein levels in each cell lysate. Green fluorescence strain DCFH-DA was used to detect ROS levels in the cell lysates following the previous report (Othman et al.

2021a). In addition, lipid peroxidation (LPO) was analyzed using the colorimetric methods of Ohkawa et al. (1979). In addition, Glutathione (GSH) protein level as an antioxidant marker was measured following Ellman (1959).

Statistical analysis

All data are expressed as mean ± standard deviation (S.D.). One-way ANOVA and post hoc Tukey's test were used to compare the results between groups. The statistical program Graph Pad Prism 9.3.1 was employed in the analysis. Significant differences were considered if P values < 0.05.

Results

Kaempferol-coated AgNPs characterization

The mean size of kaempferol-coated AgNPs was 200 nm, and the mean zeta potential was -20.2 mV, as shown in Fig. 1A, B. The FTIR analysis of nanoparticles is presented in Fig. 1C and showed that O–H group was represented by a broad peak at 3338.31 cm^{-1}. C–H stretch alkynes had an absorption peak at 2115.50 cm^{-1}. The C–O asymmetric stretch carbon compounds were expressed by a band at 1635.00 cm^{-1}. The amines' C–N stretching was expressed at the absorption peak of 1345.98 cm^{-1}. The C–O–C asymmetric stretch carbon compounds and C-X alkyl halides were also expressed at bands of 442.96, 431.39, and 490.76 cm^{-1}. These data indicate that kaempferol-coated AgNPs contain many functional groups which enhance their stabilization.

Regarding the XRD's results, Fig. 1D showed a broad pattern without fine peaks indicating the amorphous structure of the kaempferol-coated AgNPs. TEM observation is presented in Fig. 1E showing that the kaempferol-coated AgNPs were almost spherical in shape and less aggregated with particles diameter less than 200 nm.

Cytotoxic effect of kaempferol-coated AgNPs on HepG2 cells

The results of the MTT cytotoxicity assay are presented in Fig. 2A, showing that the growth of hepatocellular carcinoma HepG2 cells was inhibited by the effect of kaempferol-coated AgNPs in a concentration-dependent manner. The HepG2 cells viability was less than 5% at the concentration of 10 µm/ml, and the IC50 was reported to be 2.24 µm/

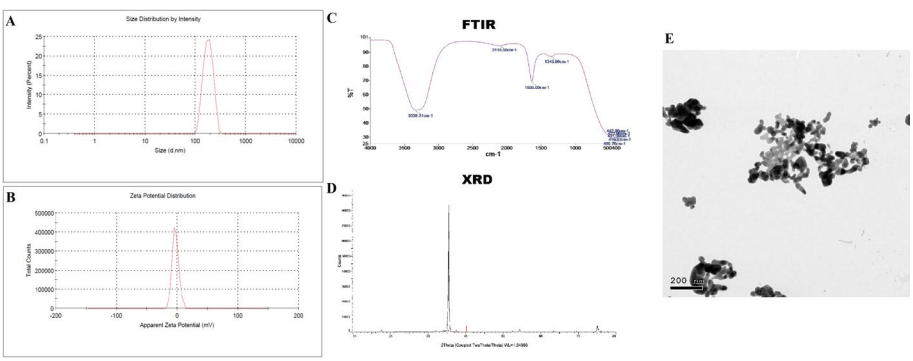

Fig. 1 Characterization of biosynthesized silver nanoparticles using kaempferol (kaempferol-coated AgNPs). **A** Hydrodynamic diameter of kaempferol-coated AgNPs as measured by Zetasizer. **B** Zeta potential of kaempferol-coated AgNPs as measured by Zetasizer. **C** FTIR spectra of kaempferol-coated AgNPs. **D** XRD pattern of kaempferol-coated AgNPs. **E** TEM image of kaempferol-coated AgNPs

ml. On the other side, the IC50 of DOX on HepG2 was reported to be 11.02 µm/ml. These data demonstrated the efficient cytotoxic effect of kaempferol-coated AgNPs on HepG2 cells using low concentrations compared to DOX drugs.

LDH leakage was also investigated to evaluate the cellular membrane leakage enhanced by the cytotoxic effect of kaempferol-coated AgNPs on HepG2 cells. LDH leakage percent is presented in Fig. 2B, showing the concentration-dependent increase in the LDH leakage, which was significantly higher than that of the control cells. DOX-induced LDH leakage percent was not significantly different from the kaempferol-coated AgNPs-induced LDH leakage. The LDH leakage percent data was in accordance with the MTT assay results confirming the cytotoxic effect of kaempferol-coated AgNPs on HepG2 cells.

Apoptotic effect of kaempferol-coated AgNPs on HepG2 cells

As shown in Fig. 3, the early and late apoptotic cells percent was significantly increased in kaempferol-coated AgNPs-treated HepG2 cells compared to the control cells and the DOX-treated cells showing the apoptotic effect of the nanoparticles.

Moreover, the protein levels of the pro-apoptotic markers (Bax, Cyt-c, P53, and caspase-3) showed significant elevations in both AgNPs- and DOX-treated cells compared to the control cells as shown in Fig. 4. While the protein level of the anti-apoptotic marker (Bcl-2) was significantly reduced by the effect of both AgNPs and DOX compared to the control cells, as shown in Fig. 4. The concentration-dependent effect of kaempferol-coated AgNPs was clear in the data as well. The apoptosis markers results revealed the kaempferol-coated AgNPs-induced apoptosis on HepG2 cells.

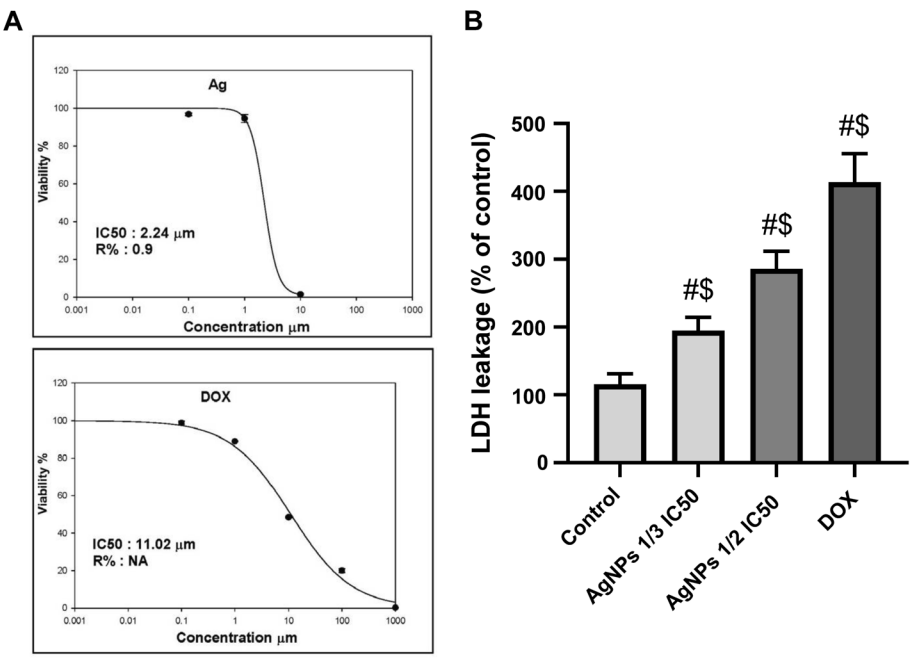

Fig. 2 Cytotoxic effects of different concentrations of kaempferol-coated AgNPs and DOX on HepG2 cells. **A** Effects of kaempferol-coated AgNPs and DOX on the viability of HepG2 cells. **B** Lactate dehydrogenase leakage percent (LDH leakage %) after exposure of HepG2 cells to kaempferol-coated AgNPs and DOX. * Indicates the significance compared to the control ($P < 0.05$)

Effect of kaempferol-coated AgNPs on HepG2 cells migration and invasion

The observations of the migration and invasion assay of HepG2 cells in the corning transwell are presented in Fig. 5. The results showed that kaempferol-coated AgNPs had an inhibitory effect to some extent on the migration and invasion of HepG2 cells. Furthermore, the same results were obtained for the DOX effect—these data demonstrate the suppressing effect of kaempferol-coated AgNPs on the migrating and invading ability of HepG2 cells.

Oxidative stress by kaempferol-coated AgNPs on HepG2 cells

To investigate whether the apoptosis effect of kaempferol-coated AgNPs is driven by inducing oxidative stress or not, ROS production expressed by DCF and lipid peroxidation (LPO) was evaluated, as shown in Fig. 6. Results showed the significant elevations of DCF and LPO by the effect of both kaempferol-treated AgNPs and DOX compared to the control cells. On the other side, the antioxidant marker (GSH) protein level was significantly reduced in cells treated with kaempferol-treated AgNPs and DOX compared to the control cells, as shown in Fig. 6. There were no significant differences between the effect of $1/2IC_{50}$ of AgNPs and DOX on both LPO and GSH levels. These data demonstrated that the kaempferol-coated AgNPs could induce the apoptosis of HepG2 cells by inducing oxidative stress.

Discussion

Cancer is one of the most death-leading diseases worldwide, and the existence of the problem is still challenging because of the induced resistance against the commonly used chemotherapeutic drugs (Geretto et al. 2017). Therefore, using novel drugs to improve the pharmacological effects on the cellular responses is recommended to

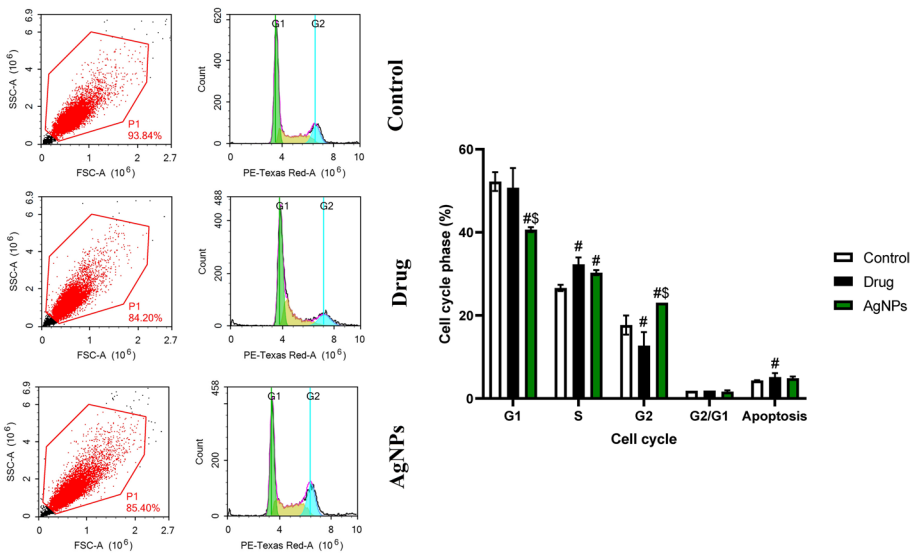

Fig. 3 Apoptotic effect of kaempferol-coated AgNPs and DOX on HepG2 cells**.** Left side shows the quadrant diagrams of HepG2 cells after staining by Annexin V-FITC and P.I. detection kit (**A** HepG2 cells without treatment, **B** HepG2 cells treated with DOX and **C** HepG2 cells treated with kaempferol-coated AgNPs). Right side shows the percentage of apoptotic cells. Values represent the mean of three experiments and analysis was done by flow cytometry. * Indicates the significance compared to the control ($P < 0.05$)

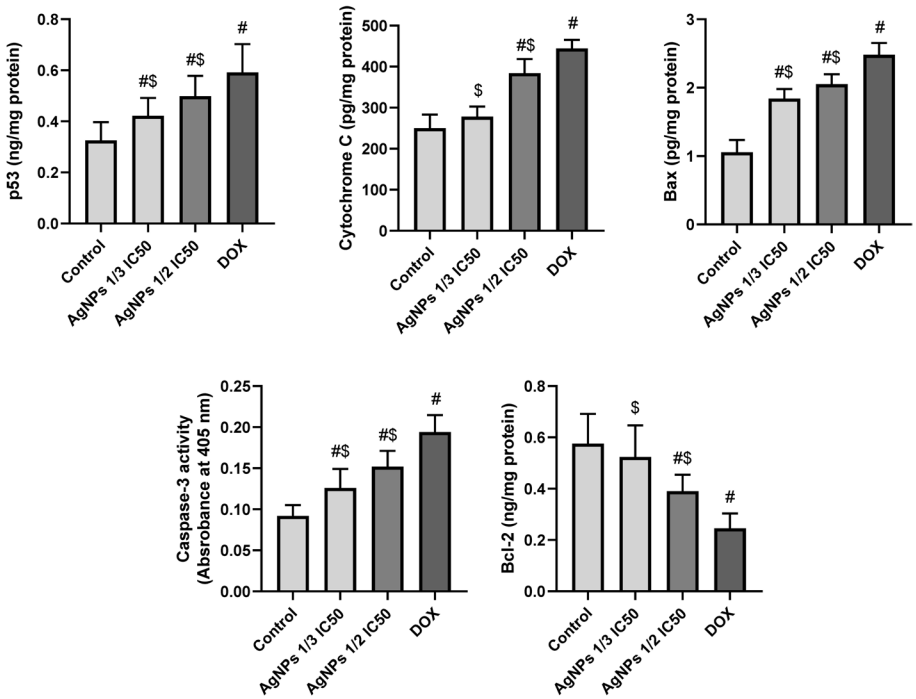

Fig. 4 Effects of kaempferol-coated AgNPs at its 1/3 and 1/2 IC_{50} doses and DOX treatment on the protein levels of apoptotic markers in HepG2 cells. Pro-apoptotic markers (P53, Cyt-c, Bax, and caspase-3), and anti-apoptotic marker (Bcl-2) were analyzed using ELISA kits. Values represent the mean of three experiments. * Indicates the significance compared to the control ($P < 0.05$)

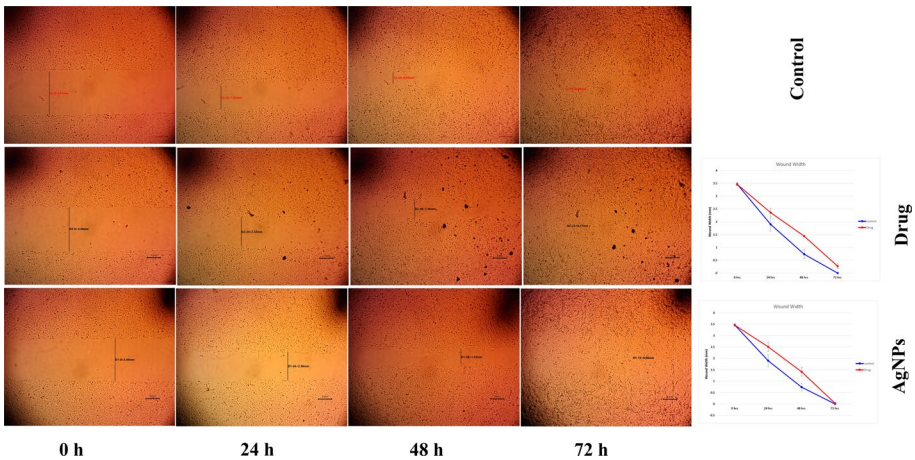

Fig. 5 HepG2 cells migration and invasion after the treatments by kaempferol-coated AgNPs and DOX at different points of time compared to the control untreated cells

avoid chemotherapy resistance (Vasan et al. 2019). Doxorubicin (DOX) is a widely used chemotherapy that fights different cancer types, such as carcinomas and sarcomas (Carvalho et al. 2009). DOX could induce cancer cells death in many pathways via intercalation into DNA and disturbing the DNA repair process; also, it could induce oxidative stress and cell membrane damage ending by programmed cell death

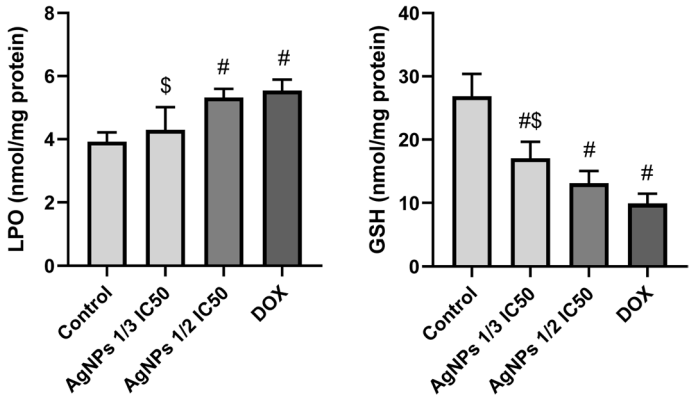

Fig. 6 Oxidative stress induced by kaempferol-coated AgNPs and DOX in HepG2 cells. ROS production expressed by DCF, lipid peroxidation (LPO) and glutathione (GSH) protein levels were measured. Values represent the mean of three experiments. * Indicates the significance compared to the control ($P < 0.05$)

(apoptosis). However, the downside of using DOX is its toxicity that can damage normal tissues, such as the heart, liver, and kidney, limiting the used dosage of the drug (Ansari et al. 2017; Carvalho et al. 2009). Therefore, in this study, we targeted the synthesis of the plant-based drug to get the desired anticancer effect with minimal adverse effects. Furthermore, since it was reported that both AgNPs and kaempferol separately could induce oxidative stress and cancer cell death, we hypothesized that the biosynthesis of AgNPs using kaempferol as a coating agent would show a synergistic anticancer efficiency.

To investigate the current hypothesis's validity, we first explored the cytotoxic effect of kaempferol-coated AgNPs and compared it to the DOX effect in HepG2 cells. Our results showed that the IC50 of the kaempferol-coated AgNPs to be 2.24 µm/ml, while the IC50 of DOX was 11.02 µm/ml, and the lower IC50 in toxicological and pharmacological known to be a more potent drug with higher safety (Meyer et al. 2019). Therefore, the used concentration of kaempferol-coated AgNPs will be lower than and safer than DOX usage to get the efficient effect. The current cytotoxic effect of kaempferol-coated AgNPs was consistent with the previously reported cytotoxic effects of seaweed-based AgNPs and walnut-based AgNPs in lung and breast cancer lines (Khorrami et al. 2018; Zhang et al. 2020). Furthermore, green synthesis of AgNPs using phytochemicals as reducing and capping agents has been linked to increased anticancer efficacy in previous studies (Khan et al. 2021; Othman et al. 2022; Wypij et al. 2021). The cytotoxic effect of biosynthesized AgNPs in this study was confirmed by evaluating the LDH leakage percentage from the damaged cells, commonly used as a cytotoxicity indicator that affects cellular membrane integrity (Han et al. 2011). Our data confirmed the concentration-dependent effect of kaempferol-coated AgNPs, which was in the same significance as the DOX effect on the LDH leakage percentage in HepG2 cells. The enhanced release of LDH by the action of 100 nm AgNPs was studied in a previous report (Kim et al. 2012) and could be explained by the effect of the release of the silver ions which induce the cytotoxic effect via metallothionines formation and genes expression disturbing (Foldbjerg et al. 2012). In addition, it might be related to the cellular uptake of AgNPs in a nanoparticle form which was reported that it is higher than the ions released in both

lung normal and cancer cell lines via endocytosis and affecting the cellular metabolism (Cronholm et al. 2013).

In the same way, the ions are released in the cytoplasmic environment of cells, ROS release would be enhanced and induce oxidative stress. The oxidative stress was investigated in this study by evaluating the ROS production (expressed by DCF level) and lipid peroxidation level; the current results showed the concentration-dependent oxidative damage induced by kaempferol-coated AgNPs with the consumption of the antioxidant glutathione as happened by the effect of DOX in HepG2 cells. The induced ROS production and low levels of GSH were reported before in human Chang hepatic cells after their exposure to AgNPs (Piao et al. 2011). The size of AgNPs plays an important role in their mode of action, because it affects the cellular uptake of AgNPs. It has been reported that smaller NPs with their larger surface area could dissolute easier and release the ions (Johnston et al. 2010). While the coated 200 nm AgNPs could be up taken by the cells in a higher level by the endocytosis, inducing different molecular mechanisms of the cellular response (Fehaid and Taniguchi 2019). Therefore, investigating intracellular ROS generation is an important indicator of NPs-induced toxicity and could be considered the first event in the toxicity cascades (Lee et al. 2011). In case of high ROS release by the effect of NPs on cells, as happened in this study, cells eventually go to programmed cell death (apoptosis) because of the exhaustion of antioxidant (GSH) capacity of cells (Li et al. 2021). Thus, apoptotic markers (Bax, Cyt-c, P53, and caspase-3) and anti-apoptotic marker (Bcl-2) were evaluated after exposure of HepG2 cells to kaempferol-coated AgNPs. Our findings revealed that kaempferol-coated AgNPs induced apoptosis of HepG2 cells showing the anti-cancer effect by decreasing Bcl-2 and increasing Bax and Cyc-c levels that activating caspase-3, which is triggered due to disruption of membrane of mitochondria, and promoting p53-mediated cell cycle arrest. These findings are in line with a previous study that concluded that beet root-based AgNPs could induce apoptosis in human hematoma derived cell line (Bin-Jumah et al. 2020) confirming the potential anti-cancer effect of green-synthesized AgNPs.

Regarding the kaempferol effect, it was reported that it could induce cell cycle arrest and p53 phosphorylation-mediated apoptosis in the human breast carcinoma cell line (Choi and Ahn 2008). It was also recommended to use both DOX and kaempferol as a combination therapy because of their ability to amplify the toxic effect and the oxidative damage-induced apoptosis in glioblastoma cells (Sharma et al. 2007). Interestingly, in the current study, kaempferol could show a synergistic anticancer effect along with the AgNPs via oxidative stress-mediated apoptosis of HepG2 cells.

Cancer cells can spread throughout the body via metastasis which increases the mortality rate in cancer patients with poor prognosis in most cases (Tian et al. 2020). Most anti-cancer drugs focus on inhibiting cell proliferation and inducing cell death; however, targeting cancer cells' migrating and invading ability is a good approach in cancer therapy (Levin 2005). Therefore, in this study, we investigated the effect of the kaempferol-coated AgNPs on the migration and invasion of HepG2 cells. Our findings demonstrated that kaempferol-coated AgNPs could suppress the migrating and invading ability of HepG2 cells to some extent which was not much different from the DOX effect. This data supported a previous report that concluded the antimetastatic effect of plant-based AgNPs in breast and colorectal cell lines (Khan et al. 2021). On the other hand,

kaempferol itself could downregulate AKT and Focal adhesion kinase (FAK) transductions, inhibiting the migration of renal cell carcinoma (Hung et al. 2017). Altogether, it is clear that the combination between the bio-based kaempferol and the nano-silver can potentiate their antimetastatic effect in the HepG2 cell line.

Conclusions

This study concluded the potential anti-cancer effect of kaempferol-coated AgNPs on liver cancer (HepG2) cells via oxidative stress-mediated apoptosis and cell cycle arrest. As a result, our research sheds light on the mechanism through which AgNPs cause cellular apoptosis. It is recommended to use green-synthesized silver nanoparticles in cancer therapy instead of the chemotherapeutic drugs to get the desired effect with minimal toxic effects. Further in vivo studies should be done to support this study.

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Author contributions
NMA and RSA designed the project, performed the experiments, drafted and edited the manuscript. NMA and HMA analyzed the data, interpreted the data, drafted and edited the manuscript. HMA supplied the chemicals and reagents. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

Adegbola O, Ajayi GO (2008) Screening for gestational diabetes mellitus in Nigerian pregnant women using fifty-gram oral glucose challenge test. West Afr J Med 27:139–143

Al-Brakati A et al (2021) Possible role of Kaempferol in reversing oxidative damage, inflammation, and apoptosis-mediated cortical injury following cadmium exposure. Neurotox Res 39:198–209. https://doi.org/10.1007/s12640-020-00300-2

Al-Otaibi AM, Al-Gebaly AS, Almeer R, Albasher G, Al-Qahtani WS, Abdel Moneim AE (2022) Potential of green-synthesized selenium nanoparticles using apigenin in human breast cancer MCF-7 cells. Environ Sci Pollut Res Int. https://doi.org/10.1007/s11356-022-19166-2

Ansari L, Shiehzadeh F, Taherzadeh Z, Nikoofal-Sahlabadi S, Momtazi-Borojeni A, Sahebkar A (2017) The most prevalent side effects of pegylated liposomal doxorubicin monotherapy in women with metastatic breast cancer: a systematic. Rev Clin Trials 24:189–193

Balogh J et al (2016) Hepatocellular carcinoma: a review. J Hepatocell Carcinoma 3:41–53. https://doi.org/10.2147/jhc.S61146

Bin-Jumah M, Al-Abdan M, Albasher G, Alarifi S (2020) Effects of green silver nanoparticles on apoptosis and oxidative stress in normal and cancerous human hepatic cells in vitro. Int J Nanomed 15:1537–1548. https://doi.org/10.2147/ijn.S239861

Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann M-C (2005) In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol Sci 88:412–419. https://doi.org/10.1093/toxsci/kfi256%JToxicologicalSciences

Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI (2009) Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem 16:3267–3285. https://doi.org/10.2174/092986709788803312
Choi EJ, Ahn WS (2008) Kaempferol induced the apoptosis via cell cycle arrest in human breast cancer MDA-MB-453 cells. Nutr Res Pract 2:322–325. https://doi.org/10.4162/nrp.2008.2.4.322
Cronholm P et al (2013) Intracellular Uptake and Toxicity of Ag and CuO nanoparticles: a comparison between nanoparticles and their corresponding metal. Ions 9:970–982
De Stefani E et al (1999) Dietary antioxidants and lung cancer risk: a case-control study in uruguay. Cells 34:100–110
Ellman GL (1959) Tissue sulfhydryl groups. Arch Med 82:70–77
Faedmaleki F, Salarian AA, Ahmadi AH, Rastegar H (2014) Toxicity Effect of Silver Nanoparticles on Mice Liver Primary Cell Culture and HepG2 Cell Line Iran. J Pharm Res 13:235–242
Fehaid A, Taniguchi AJ (2019) Size-dependent effect of silver nanoparticles on the tumor necrosis factor α-induced DNA damage response. Int J Mol Sci 20:1038
Fehaid A, Fujii R, Sato T, Taniguchi AJ (2020) Silver nanoparticles affect the inflammatory response in a lung epithelial cell line. Cell 9:14
Firdhouse MJ, Lalitha P (2013) Biosynthesis of silver nanoparticles using the extract of alternanthera sessilis-antiproliferative effect against prostate cancer. Cells Cancer Nanotechnol 4:137–143. https://doi.org/10.1007/s12645-013-0045-4
Foldbjerg R, Irving ES, Hayashi Y, Sutherland DS, Thorsen K, Autrup H (2012) Global gene expression profiling of human lung epithelial cells after exposure to nanosilver. Gene 130:145–157
Geretto M, Pulliero A, Rosano C, Zhabayeva D, Bersimbaev R (2017) Resistance to cancer chemotherapeutic drugs is determined by pivotal microRNA regulators. Am J Cancer 7:1350
Gurunathan S, Jeong JK, Han JW, Zhang XF, Park JH, Kim JH (2015) Multidimensional effects of biologically synthesized silver nanoparticles in Helicobacter pylori, Helicobacter felis, and human lung (L132) and lung carcinoma A549 cells. Nanoscale Res Lett 10:35. https://doi.org/10.1186/s11671-015-0747-0
Han X et al (2011) Validation of an LDH assay for assessing nanoparticle toxicity. Toxicology 287:99–104. https://doi.org/10.1016/j.tox.2011.06.011
Hung T-W, Chen P-N, Wu H-C, Wu S-W, Tsai P-Y, Hsieh Y-S, Chang H-R (2017) Kaempferol Inhibits the Invasion and Migration of Renal Cancer Cells through the Downregulation of AKT and FAK Pathways. Int J Med Sci 14:984–993. https://doi.org/10.7150/ijms.20336
Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S (2010) A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed. Toxicity 40:328–346
Khan MS et al (2021) Anticancer potential of biogenic silver nanoparticles: a mechanistic study. Pharmaceutics 13:707
Khorrami S, Zarrabi A, Khaleghi M, Danaei M, Mozafari MR (2018) Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. Int J Nanomed 13:8013–8024. https://doi.org/10.2147/IJN.S189295
Kim S-H, Hwang K-A (2016) Treatment with kaempferol suppresses breast cancer cell growth caused by estrogen and triclosan in cellular and xenograft breast cancer models. Int J News 28:70–82
Kim TH, Kim M, Park HS, Shin US, Gong MS (2012) Size-dependent cell toxicity silver nanoparticles. Biomed Mater Res 100:1033–1043
Lee K, Lee H, Lee KW, Park TGJB (2011) Optical imaging of intracellular reactive oxygen species for the assessment of the cytotoxicity of nanoparticles. Biomed Mater Res 32:2556–2565
Levin EG (2005) Cancer therapy through control of cell migration. Cells 5:505–518
Li Z et al (2021) Neuroprotective effects of protocatechuic acid on sodium arsenate induced toxicity in mice: Role of oxidative stress, inflammation, and apoptosis. Int J Res 337:109392
Menon S, Shanmugam V (2020) Cytotoxicity Analysis of Biosynthesized Selenium Nanoparticles Towards A549 Lung Cancer Cell Line. J Inorg Organometal Polym Mater 30:1852–1864. https://doi.org/10.1007/s10904-019-01409-4
Menon S, Agarwal H, Kumar SR, Kumar SV (2017) Green synthesis of silver nanoparticles using medicinal plant acalypha indica leaf extracts and its application as an antioxidant and antimicrobial agent against foodborne. Int J Appl Pharm 9:42–50. https://doi.org/10.22159/ijap.2017v9i5.19464
Meyer CT et al (2019) Quantifying drug combination synergy along potency and efficacy axes. Res Durg 8:97–108
Murray CB, Kagan AC, Aroms MJ (2000) Synthesis and characterization of monodisperse nanocrystals and close-packed nanocrystal. Assemblies 30:545–610
Ohkawa H, Ohishi N (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Reaction 95:351–358
Othman MS, Al-Bagawi AH, Obeidat ST, Fareid MA, Habotta OA, Moneim AEA (2021a) Antitumor activity of zinc nanoparticles synthesized with berberine on human epithelial colorectal adenocarcinoma (Caco-2) cells through acting on Cox-2/NF-kB and p53 pathways Anticancer Agents. Med Chem. https://doi.org/10.2174/1871520621666211004115839
Othman MS, Obeidat ST, Al-Bagawi AH, Fareid MA, Fehaid A, Abdel Moneim AE (2021b) Green-synthetized selenium nanoparticles using berberine as a promising anticancer agent J. Integr Med. https://doi.org/10.1016/j.joim.2021.11.002
Othman M, Obeidat S, Al-Bagawi A, Fareid M, El-Borady O, Kassab R, Abdel Moneim A (2022) Evaluation of the potential role of silver nanoparticles loaded with berberine in improving anti-tumor efficiency. Pharm Sci 28:86–93. https://doi.org/10.34172/ps.2021.28
Piao MJ et al (2011) Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis. Toxicol Lett 201:92–100. https://doi.org/10.1016/j.toxlet.2010.12.010
Raghavan BS, Kondath S, Anantanarayanan R, Rajaram RB (2015) Kaempferol mediated synthesis of gold nanoparticles and their cytotoxic effects on MCF-7 cancer cell line. Int J Nanomed 50:1966–1976
Sharma V, Joseph C, Ghosh S, Agarwal A, Mishra MK, Sen E (2007) Kaempferol induces apoptosis in glioblastoma cells through oxidative stress. Mol Cancer Ther 6:2544. https://doi.org/10.1158/1535-7163.MCT-06-0788

Sinani VA et al (2003) Collagen coating promotes biocompatibility of semiconductor nanoparticles in stratified LBL. Films 3:1177–1182
Tang X-l et al (2015) Protective Effect of Kaempferol on LPS plus ATP-induced inflammatory response in cardiac. Fibroblasts 38:94–101
Tatsimo SJN et al (2012) Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from bryophyllum pinnatum. Cardic 5:1–6
Tian J, Wei X, Zhang W, Xu AJ (2020) Effects of selenium nanoparticles combined with radiotherapy on lung cancer. Cells 8:1289
Vasan N, Baselga J, Hyman DMJN (2019) A view on drug resistance in cancer. Cells 575:299–309
Wang S, Mamedova N, Kotov NA, Chen W (2002) Antigen/antibody Immunocomplex from CdTe nanoparticle bioconjugates. J Int Res 2:817–822
Wypij M, Jedrzejewski T, Trzcinska-Wencel J, Ostrowski M, Rai M, Golinska P (2021) Green synthesized silver nanoparticles: antibacterial and anticancer activities. Biocompatib Anal Surf Attached Proteins Front Microbiol 12:632505. https://doi.org/10.3389/fmicb.2021.632505
Zhang D et al (2020) Biosynthesized silver nanoparticles using Caulerpa taxifolia against A549 lung cancer cell line through cytotoxicity effect/morphological damage. Saudi J Biol Sci 27:3421–3427. https://doi.org/10.1016/j.sjbs.2020.09.017

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