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Galangin Enhances Gold Nanoparticles as Anti-Tumor Agents Against Ovarian Cancer Cells

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Abstract. Galangin is a flavonoid from the root of *Alpinia officinarum*. It has novel anticancer properties. In the current study, ovarian cancer cells SKOV3 were treated with galangin alone, galangin combined with Gold nanoparticles (AuNPs) and AuNPs alone, and cytotoxic activity of these agentswas investigated using MTT viability assay. The expression of apoptosis-related genes p53, caspase-8 was studied using Real time PCR assay. Galangin was tested at different concentrations. AuNPs concentrations were 6.25,12.5, 25, 50 and 100 µg/ml. The study of gene expression showed significant effects on p53, caspase8 in ovarian carcinoma cells. The results of the current study refer to galangin-AuNPs combination is synergistic against to ovarian carcinoma to induce cytotoxicity and cell death via apoptosis, this mechanism enhancing expression of p53, caspase-8. The effects of galangin, AuNPs,combination therapy have potential clinicaluse in the future and could be alternative way to the conventional chemotherapy drug.

INTRODUCTION

Women affected with ovarian cancer, which is a common malignant tumor in the world. The incidence in Asian and African women are less than white women [1]. Ovarian cancer has a low incidence in40 years old women and peaking in the late 70s, and it. Malignant serous cancers are originating from the fallopian tube mostly[2]. In Iraq, cancer incidence has elevated due to several factors related to environmental pollution associated with years of conflicts [3]. Ovarian cancer in Iraqi women is ranked seventh [4]. Ovarian cancer showed to be mostly resistant to conventional therapies [5]. So, it is very important to overcome this resistance, which needs a search for novel approaches of treatment [6].Flavonoids have various pharmacological properties and widely existed in higher plants as phenolic compounds[7-9]. Galangin is chemical substances that contain a polyphenolic compound derived primarily from different medicinal plants and its chemical structure is 4H-1-benzopyran-4-one,3,5,7-trihydroxy-2-phenyl or 3,5,7-trihydroxyflavone[10].Galangin showed to have an effect against human colon and esophageal cancer cell lines and found to induce apoptosis. Galangin also reported as anti-proliferative by inducing the suppression of expression of the proliferation-related to cell nuclear antigen (4). Nanotechnology is a unique approach for effective cancer therapy production[11, 12]. The novel chemical and physical features of nanoparticles [13], made biomedical field to use them in many applications[14] such as anti-cancer agents[15, 16]. Gold nanoparticles (AuNPs) have multiple biomedical application and found to be relatively safe [17, 18]. GNPs are tested on different and numerous numbers of cancer cell lines and was effective against many types of these cells such as breast and lung cancer cell line [19, 20] through induction of different mechanisms like the generation of oxidative stress[21]. Furthermore, AuNPs can deliver other therapeutic agents such as for loading monoterpene alcohol from aromatic plants such as linalool that reveal novel anti-breast cancer activities[22]. More useful treatment is needed, selective with high low toxicity[23]. Combination treatment found to be more effective as it

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attack cancer cells through different mechanisms [24]. In the present study, we tested AuNPs nanoparticles in combination with galangin against ovarian cancer and testing its safety on normal epithelial cells and evaluate the possible mechanism of the combination through study the mechanism of apoptosis induction.

MATERIAL AND METHOD

Cell line

SKOV3,ovarian cancer cell line, and HBL, human epithelial breast tissue was obtained from the cell Bank Unit, Department of experimental therapy, Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University/ Baghdad/Iraq.The cells maintained in10% Fetal bovine serum RPMI-1640 supplemented with, 100 units/ml of antibiotic solution (penicillin, and 100 µg/mL streptomycin) . Cells were subcultured by Trypsinization using trypsin-EDTA solution 0.25% (US Biological, USA) once a week and incubated inCo2 incubator at 37°C[25].

MTT Assay

SKOV-3 and HBL cells were seeded at 1×10^4 cells/mL in 96 well microtiter plates in complete RPMI-1640 medium. The cells were incubated overnight for attachment. GNPs, Galangin alone and combined were added in triplicate at different concentration . Then, the cells were incubated for 72 hrs. After that the cells were washed 2 times with sterilized PBS1x and then, stained with MTT stain at concentration 2mg/ml. The samples were incubated at 37ºC, after 2 hr. DMSO was added to each well the absorbance was measured at 492 nm using microplate reader[26]. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated using following equation:

Inhibition rate = A-B/A*100

where A and B are the optical density of control and the optical density of test, respectively [27].

Chou–Talalay and Combination Index

To determine synergism,the median effect (ED50) doses were measured for each agent alone SKOV3 ovarian cancer cells.Galangin and GNPs were analyzed as a non-constant ratio. Chou–Talalay combination indices (CI) for the combination of Galangin and GNPs, were measured via Compu Syn software (Combo Syn, Inc., Paramus, NJ, USA). Combination index inside 0.9 and 1.1 is described as an additive, while the combination index less than 0.9 indicate synergism. CI more than 1.1 considered antagonisms**[**28**].**

Gene Expression Assay

Apoptosis related gene expression alteration in cells treated with the AuNPs and galangin was studied usingSYBR-green real-time quantitative PCR assay. These genes were P53 and Caspase-8. The primer sets used as followings:P53 (forward:5′-CCGTCCCAAGCAATGGATG-3′), (reverse:5′-GAAGATGACAGGGGCCAGGAG-3′).Caspase-8(forward:5′-GACCACGACCTTTGAAGAGCTTC-3′),(reverse: 5′-CAGCCTCATCCGGGATATATC-3′). Each reaction consisted of 1 µl ofcDNA, 7.5 µl SYBR green, 0.3 µl Rox, zero.3 µl related primers, and the final quantity was topped up to 15µL via adding 5.6 µl of distilled water. TaqTM kit. The real-time detection of emission intensity of SYBR green reacted to double-stranded DNAs was measured through MX3005 stratagen Agilent, Germany real-time machine. The mean CT values of the selected genes were measured and used to normalize the level of expression via the ΔΔCT method. The fold change = 2ΔΔCT, ΔΔCT =ΔCT (treated target gene -Treated R16) – ΔCT (untreated target gene- untreated) [29].

STATISTICAL ANALYSIS

The data obtained in this study were statically analyzed using Graph Pad Prism 6, values were presented as the mean ± S.D of the triplicate of each experiments [30]**.**

RESULT AND DISCUSSION

Anti-Proliferative Activity of GNPs and Galangin

In this study, galangin was included to explore its effects on human ovarian cancer and normal cells enhanced by the GNPs on SKOV3and HBL cell lines. Our results suggested that galangin is antitumor agent that displayed potent cytotoxicity against ovarian cancer cells. Galangin demonstrated best synergetic combined with gold nanoparticles in most concentrations. So, combination with gold nanoparticles expanded potential therapy.IC50 of galangin-gold nanoparticles complex in SKOV3 cell line was 12.32 µg/mL and 33.14 µg/ml. To study cytotoxicity effectiveness of gold nanoparticles, galangin, gold nanoparticles +galangin against SKOV3 cell line using MTT assay to study cell viability , several concentrations of gold nanoparticles (5 µg/mL ,10 µg/mL, 25 µg/mL, 50µg/mL, 100 µg/ml) were used to treat the cancer and normal cells. The results were measured after 72 h using the MTT assay as shown in figure 1. The present study demonstrated cytotoxic effect of galangin on SKOV3 cell line figure 2 and no cytotoxic effect on Normal HBL cells figure 3, 4 respectively. The concentrations were used 31.25, 62.5, 125, 250 and 500µg/ml, IC50 = 88.4 µg/mL. IC50 of galangin combine with GNPs was 37.51 µg/mL. The effect of galangin combined with GNPs and GNPs alone on SKOV3 cell line was shown in figures 5. Effects of flavonols are difficult to prognosis, and their biological activities were potentially problematic for drug discovery labors. Galangin has unique mechanism through a relatively suppression of cyclin D3 as strong inhibitor of Hs578T cell proliferation that likely mediates this effect that targets other components of the tumor cell cycle and in situations where estrogen receptor specific therapeutics are ineffective[31]. The efficiency of the combined therapy for different doses of galangin combined with GNPs was assessed in the ovarian cancer cells SKOV3, and normal epithelial breast tissue HBL to investigate the possible enhancement of galangin to GNPs in vitro.Enhanced cytotoxicity was observed for the combination of galangin – GNPs combination at three doses figure-7a. In SKOV3, ovarian carcinoma cell line, combination therapyenhanced growth inhibition and possible interactions of galangin –AuNPs were assessed by apply equations of Chou–Talalay. When the combination index is lower than 0.9 is synergistic, while range between 0.9 and 1.1 of the combination index is additive, and less than 1.1 is antagonistic [32]. The results of the dose-oriented isobologram assay revealed synergism between galangin –GNPs, (figure 6b), where there is synergism in three points of combination (CI: 0.76088), 4 (CI: 0.52370), and 5 (CI: 0.35951) (figure 6C).The proposed anti-cancer effect is suggested to overcoming resistance to conventional cancer therapies.

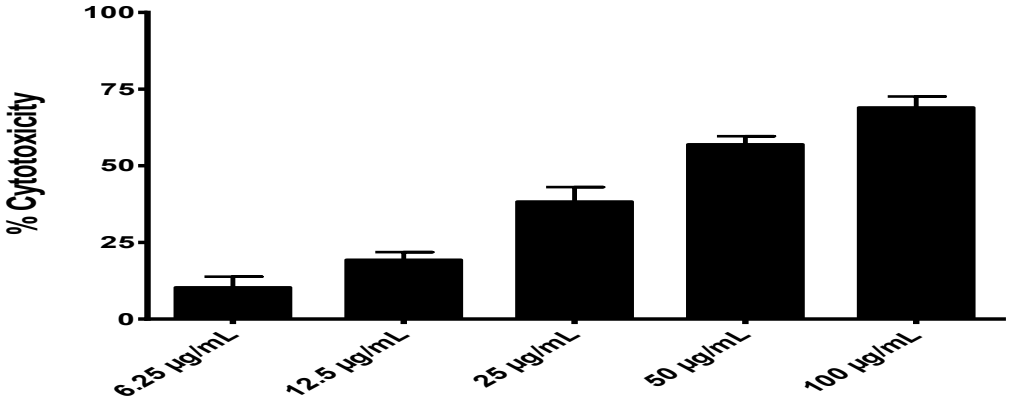

FIGURE 1. Anti-cancer activity of Gold nanoparticles in SKOV-3 cells.

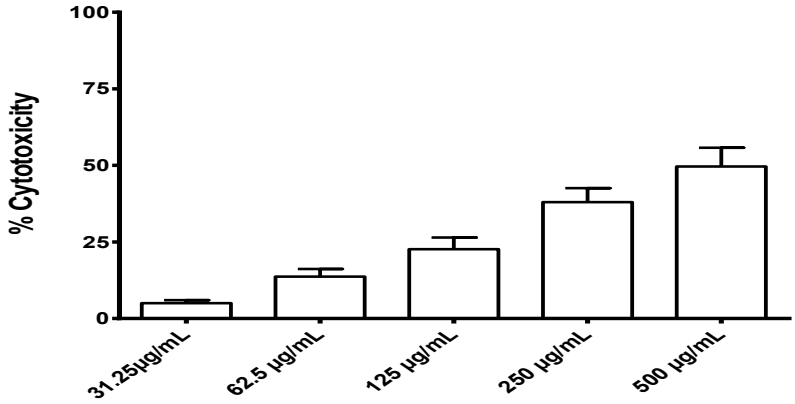

FIGURE 2. Anti-cancer activity of galangin in SKOV-3 cells**.**

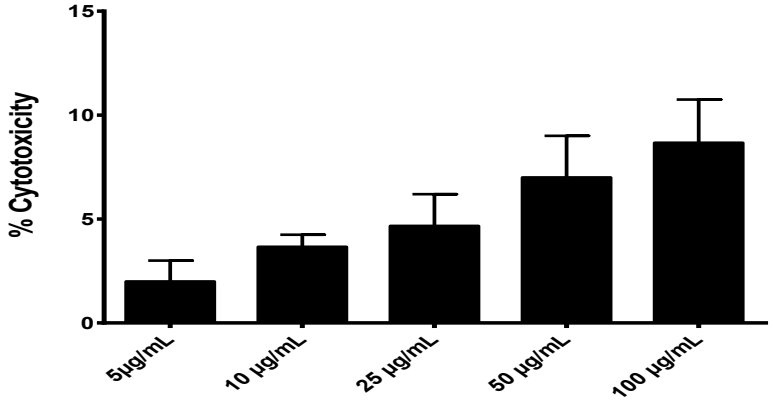

FIGURE 3. Anti-cancer activity of Gold nanoparticles in HBL cells.

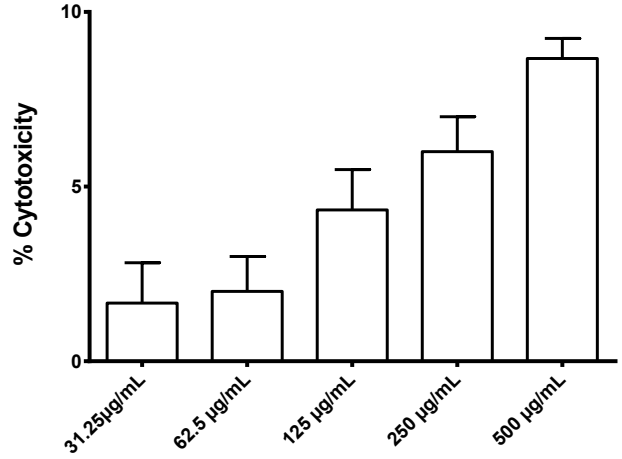

FIGURE 4. Anti-cancer activity of galangin in HBL cells.

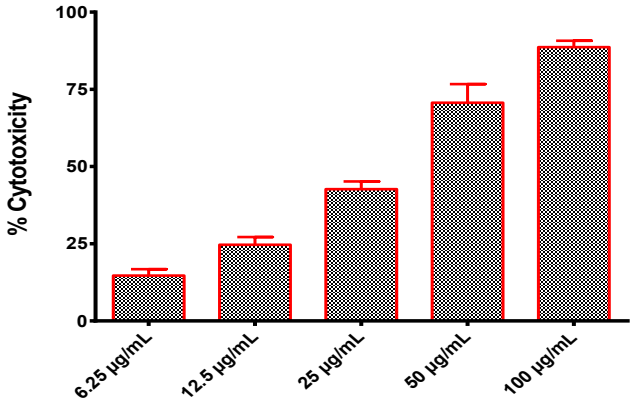

FIGURE 5. Synergistic anti cancer activity of galangin and gold nanoparticles in SKOV-3 cells.

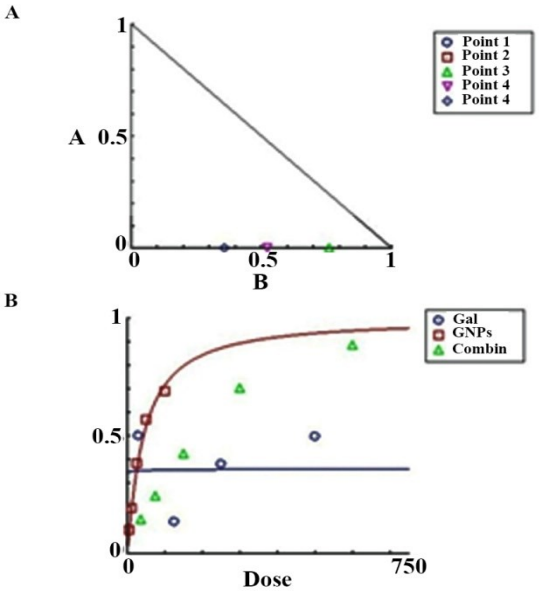

FIGURE.6. Combination index on SKOV3 ovarian carcinoma cells. A) isobologram analysis for galangin –AuNPs as the synergistic points is at the lower left of the hypotenuse. B) Dose-Effect Curve; and C) table viewing the combination index points for each combination concentration.

Galangin-Gnps Induces Cancer Cell Death Through P^{53} and Caspase-8 Pathway

In this study SKOV3 cell line were treated with galangin and gold nanoparticles and studied their effects in change of p53, and caspase-8 as shown in figures 7and 8 respectively. Our results confirmed that the p53, and caspase 8 play unimportant role in study the mechanism of how galangin, and gold nanoparticles induces apoptosis in ovarian cancer cells. Caspase 8 play crucial role for both death receptor induced apoptosis and normal T-cell proliferation of immune system [33]. Caspase-9 may block the autophagic fluxand follow blockage of cytoprotective autophagy enhancing cell death. Gold nanoparticles are less toxic than other metallic nanoparticles, its lack of clearance and more specific targeting of tumor cells. Gold nanoparticles partial rupture of the outer mitochondrial membrane trigger cell death. Gold nanoparticles targeted mitochondria of breast cancer cells and induced apoptosis elucidate an alternative application of gold nanoparticles in photothermal therapy of cancer [34]. Gold nanoparticles induce apoptosis in SKOV3 cells via p53,bax/bcl-2 and caspase 8 pathways.

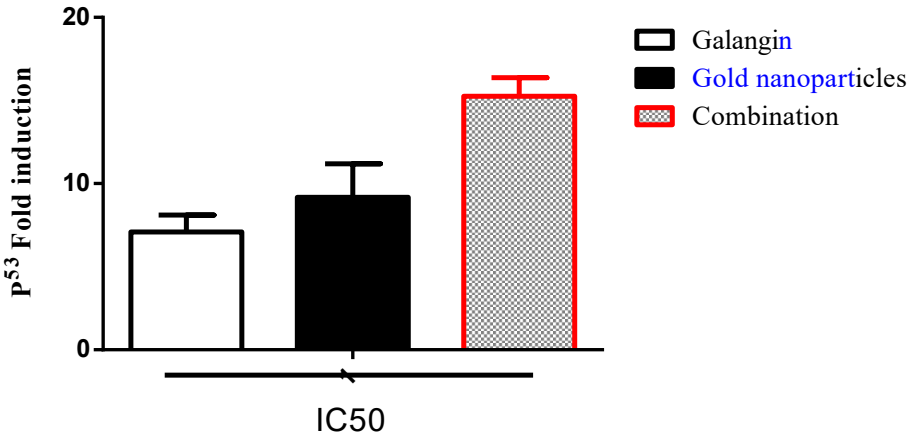

FIGURE 7. Effect of galangin and gold nanoparticles on P^{53} gene expression in SKOV-3 cells.

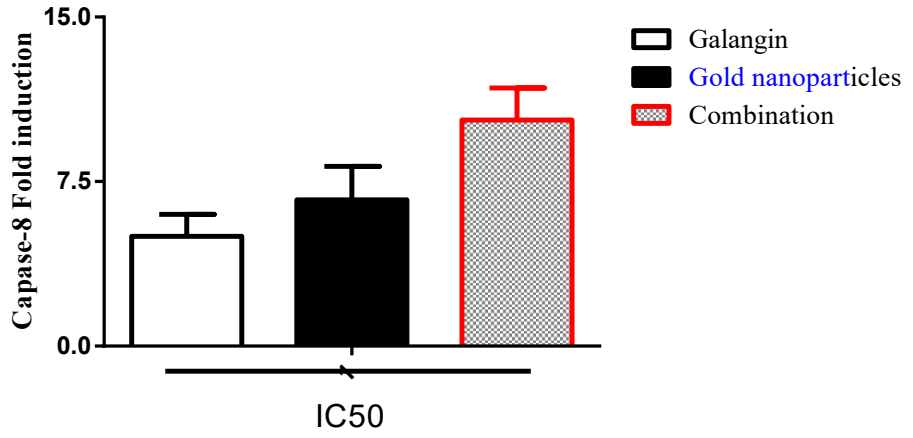

FIGURE 8. Effect of galangin and gold nanoparticles on Caspase-8 gene expression in SKOV-3 cells.

CONCLUSIONS

In conclusion, the results of current study suggested that the galangin therapeutic development as synergistic with gold nanoparticles against human breast cancer cell line (SKOV-3) through induction apoptosis illustrated biological mechanism enhancing expression p53, caspase-8 respect therapeutic targets via mitochondria pathway.

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