Galangin and Gold nanoparticles as novel anti-tumor therapy via induction of apoptosis in MCF-7 and AMN-3 cell lines

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Abstract

Galangin (3, 5, 7-trihydroxyflavone) is a flavonoid from the root of Alpinia officinarum. It has anti breast cancer properties. In the current study, AMN3 and AMF-7 were treated with galangin alone, galangin combined with Gold nanoparticles (GNPs) and GNPs alone and anti-proliferative activity of these substances was studied using MTT assay. The expression of p53, caspase-8 and caspase-9 genes was investigated using PCR technique. Galangin concentrations were used at different concentration 5, 10, 25, 31.25, 62.5, 100, 125, 250 and 500 µg/mL. GNPs concentrations was 6.25,12.5, 25, 50 and 100 µg/mL. The results of gene expression showed significant effects on p53, caspase 8 and caspase 9 in MCF-7 cell line, and AMN-3. Galangin combined with gold nanoparticles exhibited the best IC50 on MCF-7 and AMN3 with strong gene expression of p53, caspase-8 and caspase-9.

The results of current study refer to that galangin is synergistic with GNPs nanoparticles in breast cancer inducing apoptosis, this mechanism enhancing expression p53, caspase-8, caspase-9 through mitochondria pathway. The effects of galangin, GNPs, and combined between them might be used for clinical applications in future and provide new drug recompense a chemotherapy drug.

Keywords: Galangin, gold nanoparticles, p53, caspase-8, caspase-9.

Introduction

An intrinsic evidence from human, animal and cell line has been studied for traditional medicine[42]. Women affected with breast cancer are having most common malignant tumor in the world. Fertility and sexual function of young breast cancer patients are influenced with adjuvant chemotherapy[2]. Stem cells in their ability initiate and propagate metastatic tumors. Developing new therapeutic strategies to treat and prevent metastatic disease, needed understanding metastasis initiation and progression[16].

Cancer drug resistance is a complex phenomenon that is influenced by drug target alteration, drug efflux drug inactivation, DNA damage repair, cell death inhibition, inherent cell heterogeneity, epigenetic effects. It necessitates further research and treatment development[10]. Further developments in the use of atypical natural products are based on natural products in drug discovery[8].Scientific progress has failed to improve the new drug discovery[14]. *Linn.* was used for anti-fungal, anti-tumor, disease of heart, antihelmintic, anti-diuretic, anti-ulcerative, rheumatic pains, dyspepsia, fever, diabetes, chest pain, burning of liver and kidney disease[41]. Flavonoids widely existed in higher plants as phenolic compounds have various pharmacological properties. Galangin is used for oesophageal cancer in Eca9706, TE-1 and EC109 cell lines[33]. Galangin is non-toxic bioflavonoid and may be useful as a chemotherapeutic, that is combination for target components of the tumor cell cycle [29].

Galangin may be useful for human colon cancer including apoptosis[7].Galangin (GA) as antiproliferative activity is including the suppression of proliferating cell nuclear antigen expression[19]. Recent study suggested that the specific molecular mechanism of galangin-induced cytotoxicity in human breast cancer cells is still unclear[22]. Nanotechnology predicts for many applications, within biomedical uses[4]. Caspases are cysteine aspartyl proteases and 14 family members have been identified which can target for therapeutic mediation in diseases resulting in appropriate cell death[40]. Caspases respect central components of the machinery apoptosis[37]. Caspase-8 plays a role in T-cell proliferation, lymphocyte homeostasis and suppressing immunodeficiency[20].

Caspase-9 is the initiator caspase associated with the intrinsic mitochondrial pathway of apoptosis and apoptosome activation[44].The tumor suppressor protein p53 acts as a key regulator of metabolic processes and balancing between cell death and immortality with metabolic reprogramming in cancer cells[28].

In this study, we investigated the cytotoxicity of galangin, combination of galangin with gold nanoparticles alone on two cell lines: AMN3 and MCF-7, so effects on p53, caspase 8 and caspase 9 were studied.

Material and Methods

Maintenance of cell cultures: This study was carried out in the Iraqi Center of Cancer and Medical Genetic Research (ICCMGR) during October 2017– April 2018. MCF-7 cell line Michigan Cancer Foundation-7[21].and AMN3 cell line, mouse mammary adenocarcinoma were obtained from the Iraq biotech Cell Bank Unit and maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were

passaged using Trypsin-EDTA reseeded at 50% confluence twice a week and incubated at 37°C[1].

MTT assay: Galangin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). To determine the cell killing effect of galangin, GNPs (Gold Nanoparticles) and combined (galangin + GNPs), MTT assay was done. Briefly, Human Breast cancer cell lines MCF-7, and AMN-3 cell line were seeded at 10000 cells/well after 24hr or confluent monolayer is achieved, Cells were treated with galangin, GNPs, and combined (galangin+ GNPs) at different concentration as indicated. Cell viability was measured at 72 hrs of exposure by removing the medium, adding 50 µl of MTT stain and incubating for 2 hrs at 37°C. After removing the stain, add 25µl of DMSO[23]. The absorbency was determined on a microplate reader at 492. The assay was performed in triplicate.

The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

Inhibition rate = A-B/A

where A and B are the optical density of control and the optical density of test respectively.[6]

RTPCR: Gene alteration of cell line was investigated using Real-time quantitative PCR. In this experiment, three main type of gene were measured to identify the pathway of apoptosis and the mechanism action. These genes include P53, Caspase-9, and Caspase-8. Real-Time (RT)-PCR turned into accomplished to investigate the modifications in hippocampal expression genes. The primer sets have been designed based totally at the sequences from the NCBI database. The sequences of primers used within the quantitative RT-PCR assay include[5]:

P[53](forward:5′-CCGTCCCAAGCAATGGATG-3′)
(reverse:5′-GAAGATGACAGGGGCCAGGAG-3′)
Caspase-8(forward:5′-
GACCACGACCTTTGAAGAGCTTC-3′)
(reverse: 5′-CAGCCTCATCCGGGATATATC-3′)
Caspase-9 (forward: 5′-CTCTTGAGCAGTGGCTGGTC-3′)
(reverse:5′-GCTGATCTATGAGCGATACT-3′)

Each RT-PCR reaction combination containing 1 µL of cDNA, 7.5 µL SYBR green, zero.3 µL Rox, zero.3 µL related primers, and the final quantity was topped up to 15 µL via adding 5.6 µL of distilled water. The assay was performed with SYBR Premix Ex. TaqTM kit. The real-time detection of emission intensity of SYBR green reacted to double-stranded DNAs and was performed via the implemented Biosystems (ABI) Prism Sequence Detection system. GAPDH mRNA had been used as an inner control to identify the relative expression amount of the genes. The equation below is used to calculate the value of Threshold Cycle (CT) where the Reporter fluorescence is greater than the brightness at the Threshold which represents the amount of gene expression of isolates under study as follows (refer):

The fold change = $2^{\Delta\Delta CT}$, ΔΔCT =ΔCT (treated target gene - Treated R16) – ΔCT (untreated target gene- untreated R16)[27].

Statistical analysis: Data of three replicates were analyzed by t-test using Graph-Pad Prism version 5.01 and presented as mean ± S.E. $p \leq 0.05$ was considered significant.[24]

Results and Discussion

Cytotoxicity Assay: In this study, galangin was included to explore its effects on human breast cancer enhanced by the GNPs on MCF-7 and AMN3 cell lines .Our observations strongly suggested that galangin is a potential antitumor agent that displayed potent cytotoxicity against both as flavonoid compound showed significant cytotoxicity effect in both MCF-7 and AMN-3 at $p \leq 0.05$ and could be considered as a potential therapy, recommending its utilization in breast cancer treatment. 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 both AMN3 and MCF-7 cell line was 6.907 µg/mL and 28.32 µg/ml. To study cytotoxicity effectiveness of gold nanoparticles, galangin, gold nanoparticles +galanginon MCF-7 and AMN3 cell lines using MTT assay to study cell viability, several concentrations (5 µg/mL ,10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/ml) were used to treat the 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 MCF-7 cell line. The concentrations were used 31.25, 62.5, 125, 250 and 500 µg/ml, IC50 = 125 µg/mL as shown in figure 2. IC50 of galangin combine with GNPs was 31.25 µg/mL as shown in figure 3. The effect of galangin alone, galangin combined with GNPs and GNPs alone on AMN-3 cell line was shown as in figures 4 to 6. *Alpinia galanga* rhizome was considered as a potential chemotherapeutic agent in breast cancer, as apoptotic effects in a breast cancer cell line [35].

However, recent study suggested that naturally occurring flavonoids can revise TRPC5 channels and that one consequence in vivo might be modulation of adiponectin secretion. Effects of flavonols are difficult to prognosis, and their biological activities were potentially problematic for drug discovery labors[30]. Zhang et al[46], reported that galangin included its effects on human breast cancer, to be useful in various diseases, such as lung injury, nasopharyngeal carcinoma and melanoma development. In addition they suggested that galangin induces apoptosis of human colon cancer cells and may prove beneficial in the development of therapeutic agents for human colon cancer[7].

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. The results suggested that this non-toxic bioflavonoid, chiefly in combination with agents may be useful as a chemotherapeutic[29].

Zhang et al[47], demonstrated that galangin induces Hepatocellular Carcinoma Cells apoptosis *via* the mitochondrial pathway. Galangin has a novel pharmacological function of antimetastasis. Galangin effectively inhibited adhesion, cell proliferation, spreading, lamellipodia and motility formation *in vitro*. It is expected that galangin may serve as an efficient anti-metastasis herbal medicine for melanoma treatment[30].

Indeed, chemical mechanism for flavonoids was illustrated, the 2,3-double bond in ring C, appropriate hydroxyls, ring B attached at position 2, hydroxyls in position 3, ortho-substituting hydroxyls in ring B may be key structural requirements for potent cytotoxicity to HL-60 cells[33].This knowledge renders us at one horizon nearer to completely understanding the impacts of galangin and gold nanoparticles on the deterrence of malignancy multiplication.

Effect of galangin and GNPs on p53, caspase8 and caspase9 genes expression: Current study included stimulated MCF-7 and AMN-3 cell lines stimulated by galangin + gold nanoparticles and galangin and effects on change p53, caspase-8, and caspase-9 genes expression as shown in figures 7 to 9 respectively. Furthermore, the expression levels of p53, caspase 8 and caspase 9 important tumor suppressor proteins, were increased significantly after exposure to galangin. We strongly confirmed p53,caspase 8 and caspase 9 that play a role in disease start. The significant perceptions in improving drugs were checked in our examination. Gold nanoparticles, galangin combined with gold nanoparticles and galangin were expanding fold changes quality articulation of p53, caspase 8 and caspase 9 in both AMN3 and MCF-7 cell lines. Kim et al[15], suggested that galangin induced apoptosis of SNU-484 cells might involve changes in expression of GSTP and Uch-L1 and activation of caspase-3, -9[15].

On other hand results indicated that galangin induced both apoptosis and cell gold nanoparticles autophagy in different concentrations through p53-dependent pathway in HepG2 cells. These findings may help in the discovery of the novel pattern of treatment of hepatocellular carcinoma cells by inhibiting cell proliferation[43]. p53 activity is controlled by ubiquitination which is complex, however recent studies have shown that p53 is stabilized but still degraded in the cells of Mdm2-null mice[18]. Our investigation proposed galangin gold nanoparticles impact on human breast development induced autophagy and apoptosis through increased high expression the p53, caspase - 8, and caspase - 9 via mitochondria pathway which may show significance in the progression of remedial administrators for the treatment of human breast cancer.

Our study disagreed with Pu et al[32] that confirmed caspase-8 expression is not associated with breast cancer-specific survival. Caspase 8 play crucial role for both death receptor-induced apoptosis and normal T-cell proliferation of immune system[30]. Caspase-9 may block the autophagic flux and follow blockage of cytoprotective autophagy enhancing cell death[12].

Gold nanoparticles are less toxic than other metallic nanoparticles, its lack of clearance and more specific targeting of tumor cells. The results of early clinical trials of gold nanoparticles in lung cancer patients and head and neck cancer patients will provide new insights into these particles' in breast cancer patients[17].

Gold nanoparticles conjugates were dispatched to mitochondria. 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[26]. Gold nanoparticles induce apoptosis in MCF-7cells via p53, bax/bcl-2 and caspase pathways[36].

Recent study observed biological response of gold nanoparticles as an incomplete mechanistic understanding[34]. Scientists introduced a process -poly-ethylene glycol (PEG) layer called "PEGylation". In this approach, nanoparticles "hide" by masking their surface. This saves them from immune recognition in essence prolonging their blood circulation[39].

In addition, gold nanoparticles can easily permeate tumor vasculature and remain in tumors owing to the enhanced permeability and retention (EPR) effect[13].Many recent studies have shown promotive results using nanomedicine to target tumor microenvironment vasculature, extracellular matrix and immune response. Nanoparticles proposed a potential solution to all of these obstacles[11,38].

In conclusion, the results of current study suggested that the galangin therapeutic development as synergistic with gold nanoparticles against breast cancer in human and mouse during induction apoptosis illustrated biological mechanism enhancing expression p53, caspase-8, caspase-9 respect therapeutic targets via mitochondria pathway.

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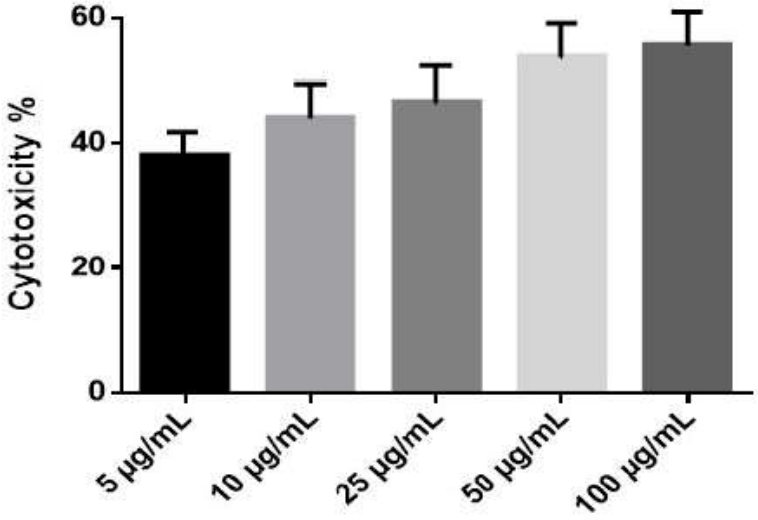

Fig. 1: Cytotoxic effect of GNPs on MCF-7 cell line

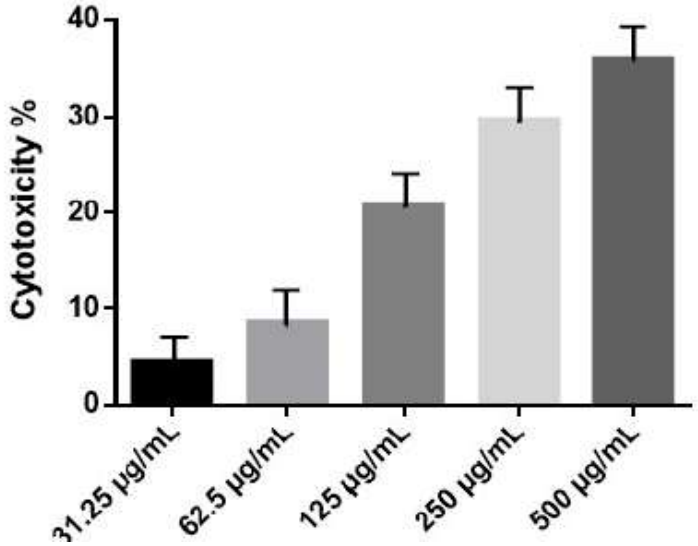

Fig. 2: Cytotoxic effect of galangin on MCF-7 cell line

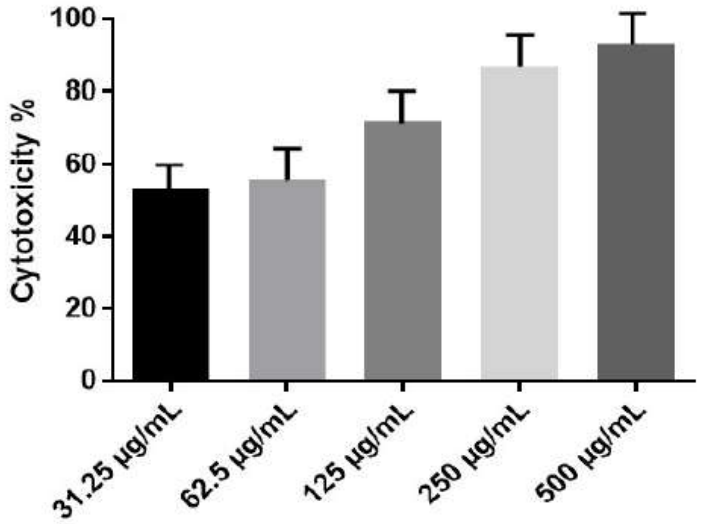

Fig. 3: Cytotoxic effect of galangin comined with Au nanoparticles on MCF-7 cell line

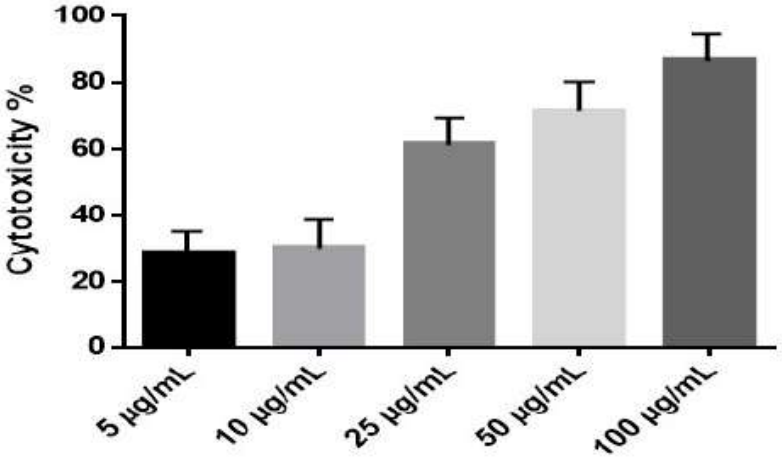

Fig. 4: Cytotoxic effect of Gold nanoparticles on AMN3 cell line

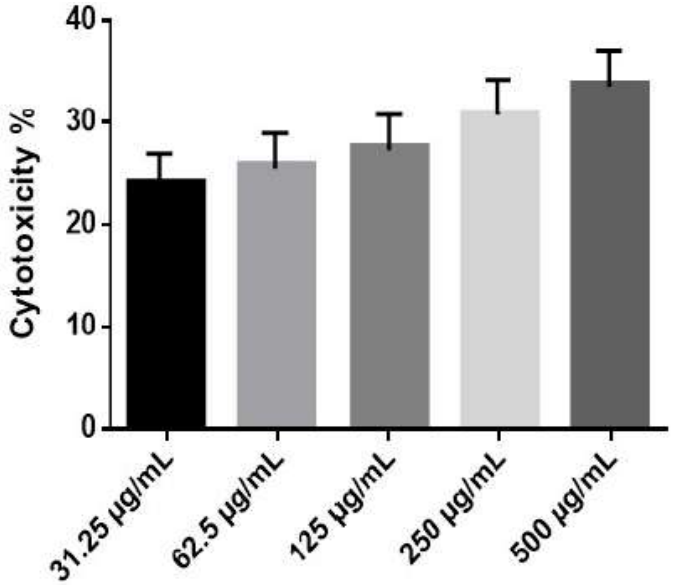

Fig. 5: Cytotoxic effect of galangin on AMN3 cell line

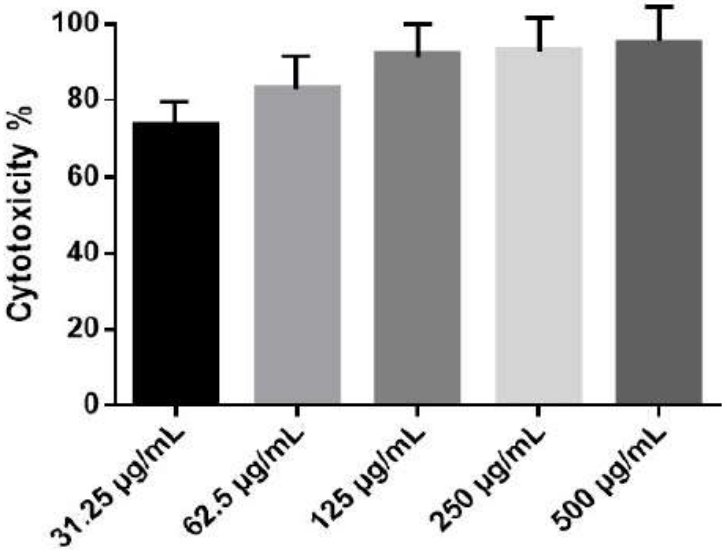

Fig. 6: Cytotoxic effect of GNPs+galangin on AMN3 cell line

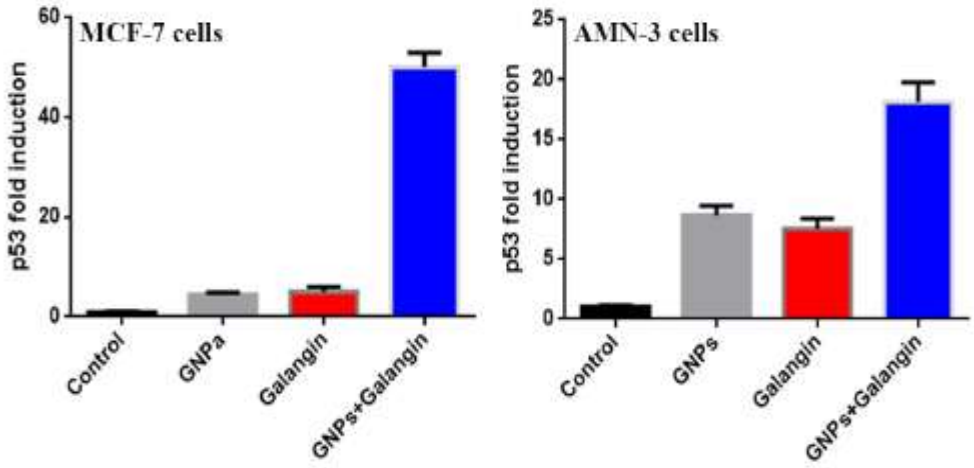

Fig. 7: Effect of GNPs, galangin and GNPs combined with galangin in p53 gene expression.

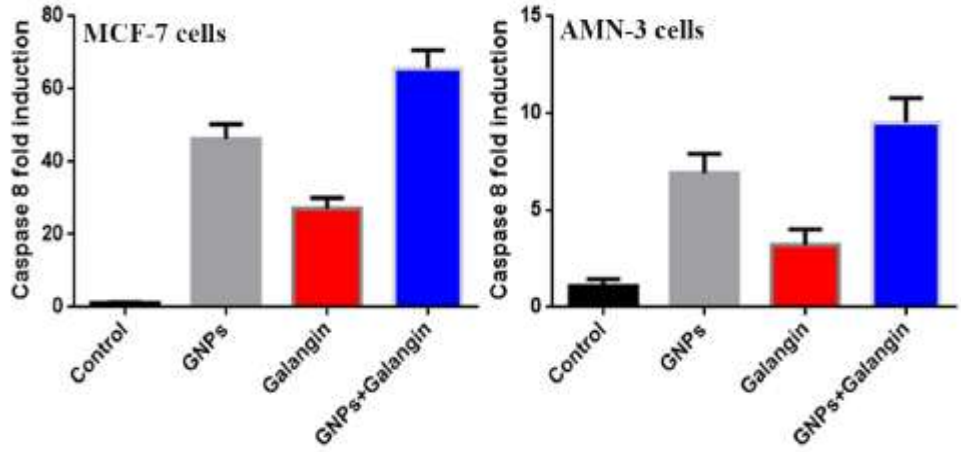

Fig. 8: Effect of GNPs, galangin and GNPs combined with galangin in caspase -8 gene expression

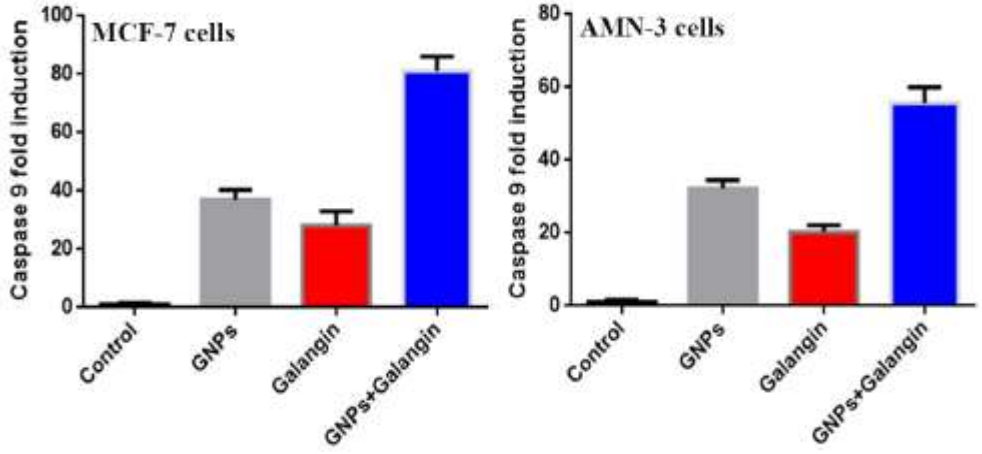

Fig. 9: Effect of GNPs, galangin and GNPs combined with galangin in caspase -9 gene expression

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