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ANTICANCER EFFECTS OF COMBINED PHOTOBIOMODULATION, CARBOPLATIN, *CASSIA FISTULA* SEEDS EXTRACT AND GOLD NANOPARTICLES (AUNPS) ON PROSTATE CANCER CELL LINE (PC3)

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ABSTRACT : Although, chemotherapy still the main mode for managing cancer but its usage is limited by resistance and adverse effects which are depend on the dose. Other modes or techniques such as LASER irradiation, anti-cancer plant or nano-metals particles can be used to solve this problem. In this study, we use three different agents (carboplatin, cassia fistula seed extract, and gold nanoparticles) where the cytotoxicity of them was investigated on prostate cancer (PC3) cell line. The PC3 cells were exposed to six doses for each of carboplatin, cassia fistula fruits extract, and gold nanoparticles. Then for each agent, the dose that gave significant decrease in the viability percent was used in combination with a constant dose of He-Ne laser (635 nm). From the results, we found that the inhibition percent was increased by the combination therapy as compared to the mono-therapy for each agent. Also, the highest inhibition percent was observed in the combination of gold nanoparticles and 191 J/cm^2 laser. In a conclusion, LAESR is an amazing technique to increase the cytotoxicity of the classical and non-classical methods for treating cancer especially its use in combination with gold nano-particles in the photo-thermal technique.

Key words : LASER, cancer cells, plant extract, chemotherapy, PTT.

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INTRODUCTION

The trend of cancer grows pointedly around the world. This observation indicates an inadequacy in cancer therapies, which are surgical procedures, chemotherapeutic drugs and radiotherapy. Despite the aggressiveness and the side effects of such classical treatments, the survival rates have remained unchanged. So that, there is an extreme need for developing anti-cancer therapy with fewer side effects, higher treating capacity as possible (Al Ssad *et al*, 2018).

Although, chemotherapies have been used to a great extent in the field of cancer treatment, they do not kill the cancerous cells alone but also they effect the normal cells. One of the alternative ways to replace these traditional modalities is the using of plants or herbs in the field of anti-cancer pharmacology. Plant kingdom has many plants that have excellent anti-cancer effect and one of them is *Cassia fistula*, which belong to Fabaceae family (Safwat *et al*, 2018). The extracts of *Cassia fistula* have been used for diverse pharmacological activities like wound healing properties, anti-inflammatory, antimicrobial, antioxidant, and anticancer activity.

Today, recent advanced-medical technique to treat life- threating diseaeses such as cancer are developed. Photo-thermal technique (PTT) is the most modern method for the treatment of cancer. This technique depend on the use of two important therapeutic tools, which are LASER and Nano-particles (Fekrazad *et al*, 2016).

PTT has superior property over chemotherapy which is the selective killing of cancer cells under LASER irradiation, without causing extreme damage to normal tissues providing high degree of accuracy (Cheng *et al*, 2014). Nanoparticles are favorable to be used in PTT as photothermal agents because of their unique propertie6s e.g. high photo-thermal capability and small diameters which permit tumor penetration with special localization in tumor rather than any part in the body because the blood vessels of tumors are fragile and so more leaky

than normal blood vessels (Vines *et al*, 2019). Gold nanoparticles are the most photo-thermal agent to be used in PTT because they have extreme power to absorb LASER light, non-toxic to the *in vivo* environment and have tunable optical characteristics (Her *et al*, 2017).

Prostate cancer is the second most frequent diagnosised-cancer in men and the fifth leading cause of death worldwide. Most patients with this cancer are already in advanced- stage at the time of diagnosis. The options of treatment for prostate cancer involve: surgery, chemotherapy, radiation, targeted therapy (prostate specific membrane- antigen) and combination therapies. Although chemotherapy is the major therapeutic option for prostate cancer but this choice has limited because they cause severe adverse effects due to their non-selectivity towards cancerous cells. So that, other modalities such as using natural plant extract or using nano-photothermal therapy may decrease the problems that occurs with chemotherapy in the management of prostate cancer (Thambiraj *et al*, 2018).

MATERIALS AND METHODS

Experiment design : Prostate cancer cell line (PC3)was supplied as a gift from cell culture unit in Babylon medical college. The cell was seeded at 4X10 with RPMI-1640 culture media with 10% FBS and 1% penicillin-streptomycin antibiotics and incubated at 37°C. For determination of toxicity range, the cell was seeded in a 96 well plate for 24h or until monolayer was reached to 80-90% and exposed to serial dilutions of carboplatin, cassia fistula extract, and gold nanoparticles to screen the dose-response. After experiments the does toxicity, has been decided and six concentrations range of each agent was tested. Carboplatin (31.25, 62.5, 125, 250, 500, 1000) µg/ml, *Cassia fistula* (31.25, 62.5, 125, 250, 500, 1000) µg/ml, and gold nanoparticles (50, 100, 200, 300, 400, 500) µg/ml. Then, three effective concentrations of each agent were used in combination with 635 nm laser on PC3 cell line.

Exposure of PC3 cells to He–Ne laser

The prostate cancer cell was plated on 96 well tissue culture plates and exposed to six dose concentrations of each agent. At the same time the plate wells with the cancer cell was exposed to continuous TEM00 mode He–Ne laser radiation with beam profile at a wavelength of 635 nm and 300 mW output power. The laser was centered vertically upon each well at a distance of 10 cm from the bottom of the plate, and 1.1 cm^2 radiotion area. For avoiding accidental radiation errors, the wells that was included for radiation was covered at each application session (Ghaleb *et al*, 2014).

Preparation of *Cassia fistula* extract

The fruits was dried in a dark room for 2 weeks and grinded into fine powder. 40 g of dried powder was soaked in 200 mL D.W for 24 h, and then filtered by using Whatman filter paper and concentrated by evaporation under vacuum reduced pressure at 45°C for 30 min using a rotary evaporator.

Crystal violet assays

After the end of the experiment, the old media with the agents was taken out by a micropipette and each well was washed by PBS, Prostate cancer cells were fixed with 10% formalin at room temperature for 20 minutes solution was removed. 100 µl of 0.1% aqueous crystal violet solution was added to each well and removed after 20 minutes exposure. The plate then washed by immersing smoothly in a tap-water container many times until the water become clear. The plate was dried and 200 µl of 95% ethanol was added for each well. The optical density (OD) of the plate wells was recorded at a wavelength 540 nm. The results showed after been normalized to the control.

Determination of IC50 for cell lines

IC50-data were determined graphically by plotting the 50% of maximal inhibition for each time of exposure and period of incubation on the inhibition- concentrations curve.

Statistical analysis

Data were expressed as the (mean ± SEM) and the differences using one-way ANOVA. Statistical analyses were performed by SPSS software followed by the graph pad prims program. P-value of <0.05 was reflected as a significant difference.

RESULTS AND DISCUSSION

Each agent in this study was used in six concentrations. For AuNPS, only the three highest concentrations showed a significant decrease in the viability percent for PC3 cells as compared with the control group (Fig. 1).

The IC50 for AuNPs, Cassia fistula and Carboplatin was calculated as represented in Figs. 2, 4, 6, respectively.

Also, the three highest concentrations of cassia fistula showed a significant decrease in the viability percent of PC3 cells as compared with the control cells (Fig. 3).

Carboplatin showed a significant decrease in the viability percent of PC3 cells for all concentrations except with the lowest one which showed a non-significant decrease in the viability percent of PC3 cells as compared with the control cells (Fig. 5).

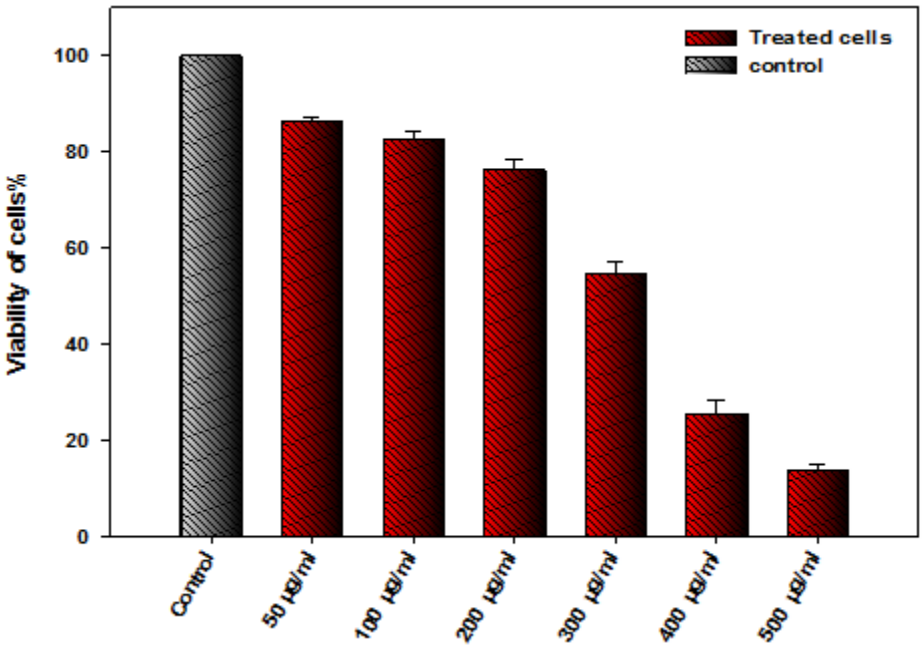

Fig. 1 : The effect of AuNPS on PC3 Cell line.

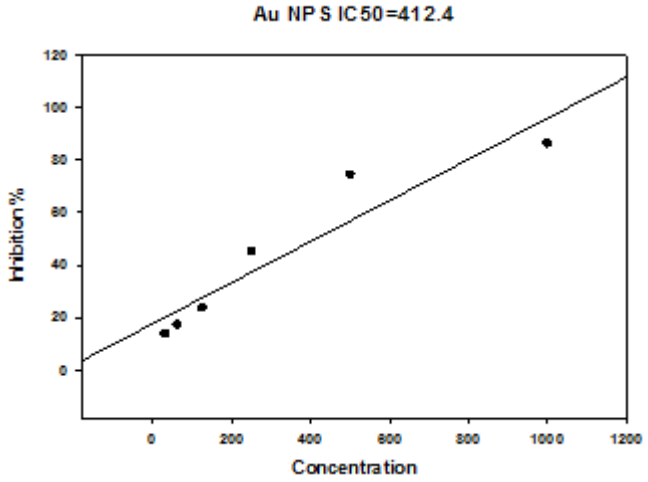

Fig. 2 : Dose-Response curve of growth inhibition % of gold nanoparticles presented by plotting of concentration versus GI% values.

The three highest concentrations (1000, 500, 250) µg/ml of cassia fistula extract when combined with LASER therapy showed inhibition percent of PC3 cells more than the plant extract alone and this result appeared to be clear in Fig. 7.

The combination of carboplatin and LASER (635nm) showed an increase in the inhibition percent of PC3 cells as compared with carboplatin alone, but the lowest concentration (31.25 µg/ml) remained to give a non-significant decrease in the viability percent of PC3 cells despite its combination with LASER therapy.

The combination of AuNPs with concentrations (300, 400 and 500µg/ml) and 635 nm LASER therapy showed a highly significant decrease in the viability of PC3 cells as compared with these three concentrations alone. Also, the combination of AuNPs and LASER therapy (Fig. 8) showed better results than the combined effect of Cassia fistula extract or carboplatin plus LASER therapy.

Drug resistance and dose-dependent adverse effects of traditional chemotherapies (such as platinum compounds) are the major causes of treatment failure and so relapse of cancers so that in this study we used other strategies to fight cancers such as herbal medicine and photo-thermal technique.

Using herbs-based medicines, we can avoid the side effects of conventional chemotherapy in the treatment of cancer because these anti-cancer plants can block the critical biochemical and signal transduction pathways that convert the normal cells to cancer cells (Thapliyal *et al*, 2018).

Many studies prove the activity of plant extract of *Cassia fistula* in the treatment of cancers such as prostate cancer. In this study, we noticed a more significant decrease in the viability of prostate cancer cells when we used Cassia fistula extract in combination with 635 nm LASER therapy. This result can be expected because LASER light is characterized by its coherence and collimation which provide high-intensity light with a narrow beam and so this light has great precision.

The discovery of less toxic metallic compounds (such as gold, silver, etc.) allows designing new techniques for cancer treatment (Pedrosa *et al*, 2018). PTT is the therapeutic method that enable to use light irradiation that is converted by photothermal agents (e.g. gold nano-

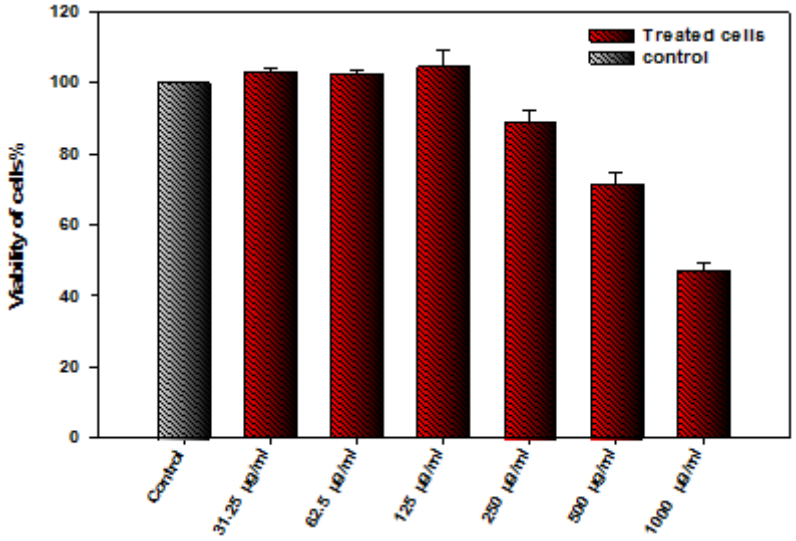

Fig. 3 : The effect of Cassia fistula extract on PC3 Cell line.

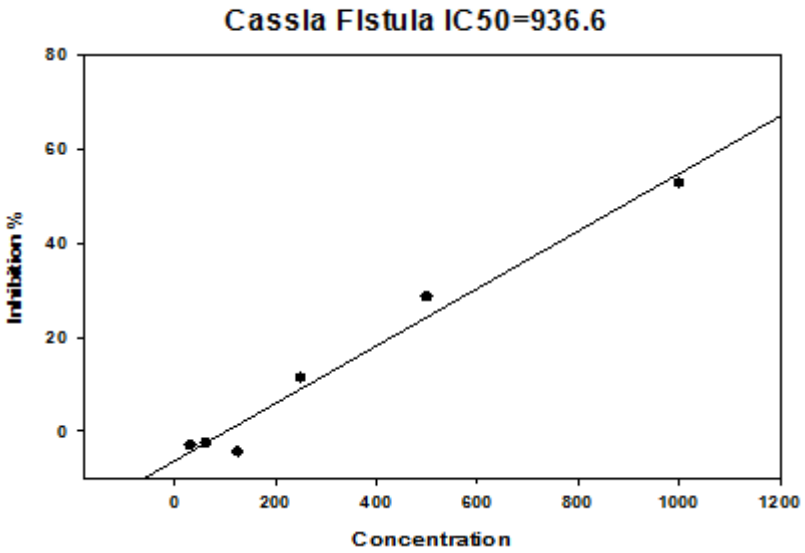

Fig. 4 : Dose-Response Curve of growth inhibition % of Cassia fistula presented by plotting of concentration versus GI%.

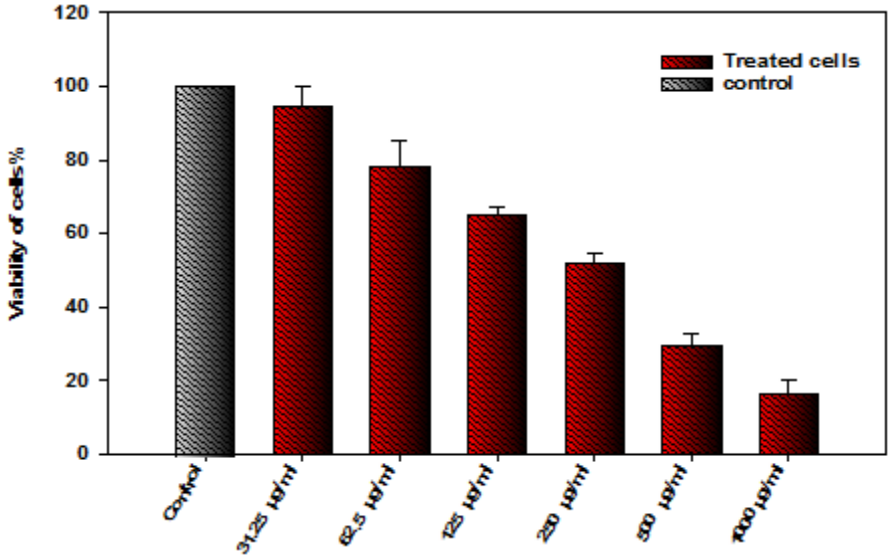

Fig. 5 : The effect of Carboplatin on PC3 Cell Line.

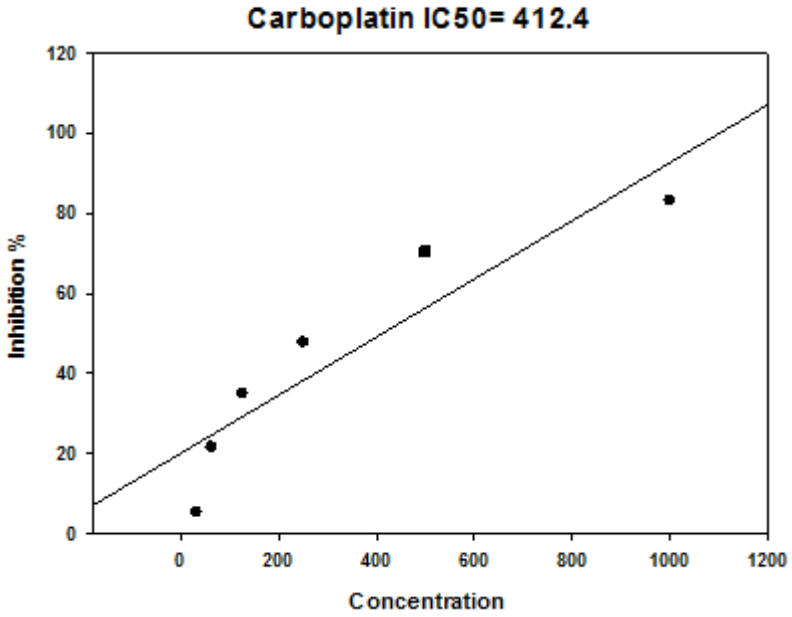

Fig. 6 : Dose-Response curve of growth inhibition % of carboplatin presented by plotting of concentration versus GI%.

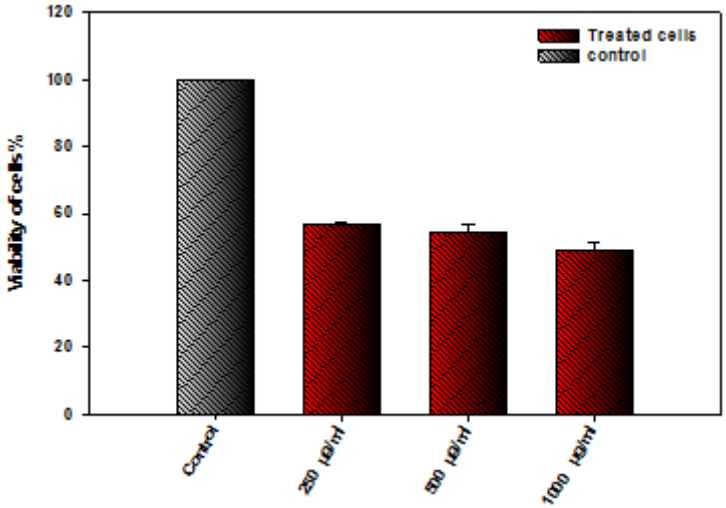

Fig. 7 : The effect of Cassia Fistula extract and 635 nm LASER on PC3 cell line.

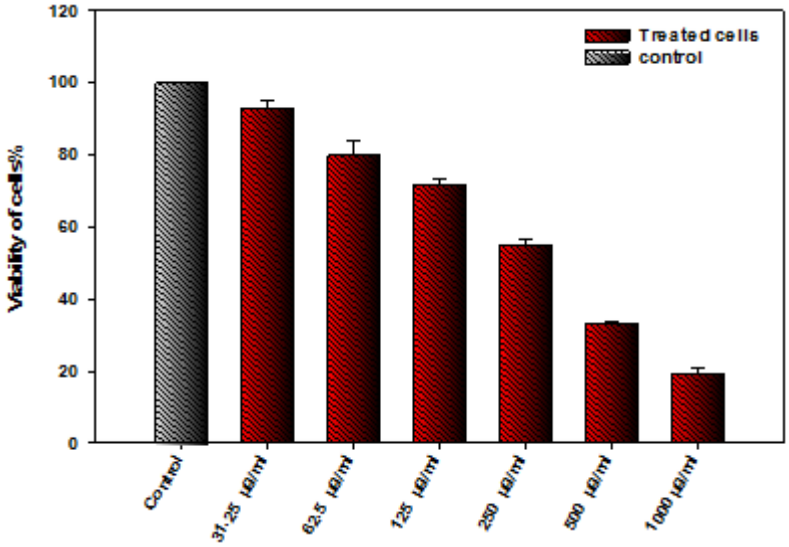

Fig. 8 : The effect of Carboplatin with 635 nm LASER on PC3 Cell line.

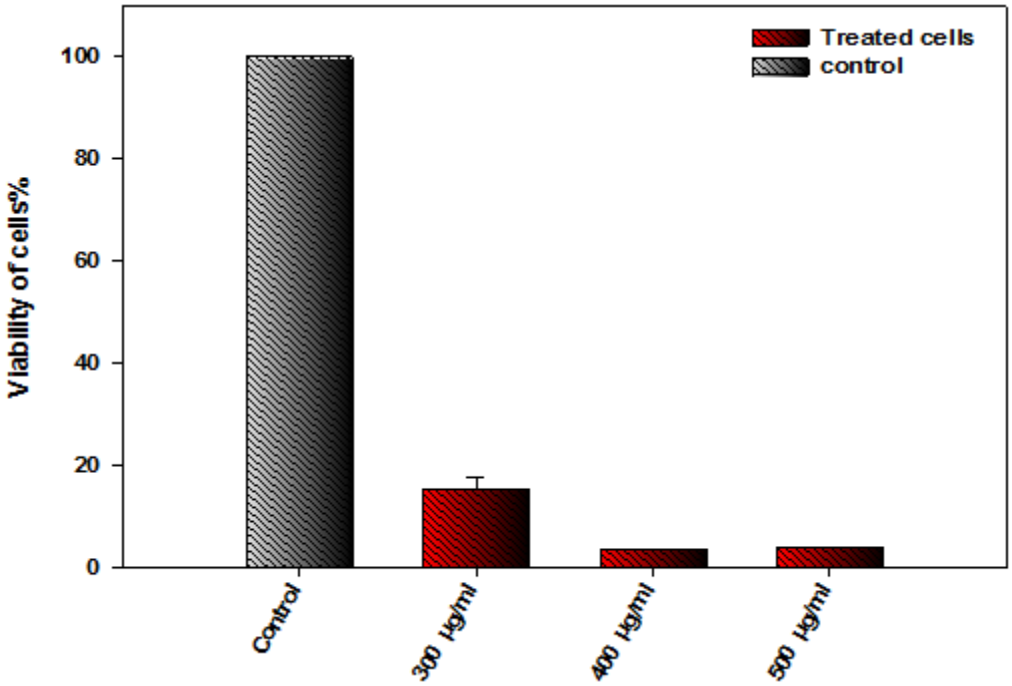

Fig. 9 : The effect of AuNPS and 635 nm LASER on PC3 Cell line.

particles) to heat, so this technique kill the cancerous tissue by increasing the temperature with little effect on the adjacent healthy tissue because the micro-environment of tumor is more acidic, hypoxic and deficient in nutritive substances than healthy tissues (Mendes *et al*, 2017; Mahal *et al*, 2019).

The cytotoxicity of carboplatin on PC3 cells increased upon irradiation of these cells with 635 nm LASER light. The result that is obtained above indicate the synergism between chemotherapy and PTT because carboplatin is a DNA- damaging agent and so its efficacy is enhanced by hyperthermia since DNA repairing- processes are temperature-dependent (Pedrosa *et al*, 2018).

From the results of this work, the best cytotoxic effect obtained from the combination of AuNPs with 635 nm LASER. This result can be explained on the bases that AuNPs can absorb light strongly and efficiently, convert photon energy into heat quickly, and all the absorbed light is converted to heat so that PTT consider as optimal treatment tool because the cancerous cells required only one third of the energy need by normal cells (Hussein *et al*, 2016).

CONCLUSION

In a conclusion, the use of LASER modality in combination with chemotherapy, anti-cancer plant or metal nanoparticles will give prodigious result in killing and targeting cancerous cells due to its unique properties especially when it is used with AuNPs in PTT and as a result, it will provide a less invasive method of cancer treatment with minimum side effects.

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