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Augmenting the Activity of Curcumin Nanoform with Laser as Anti-Cancer Against Lung Cell Line (A549)

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Abstract
Curcumin was prepared in the form of nanoparticles (CNP) using a modified technique of homogenization in an Alginate and Tween 20 (T20) matrix. The natural capsulated in nanoform was proved through TEM images which showed spherical micelles between 7 and 42 nm diameter. Using HPLC-Mass spectrum for bulk comparing to curcumin nanoform found some metabolites as; curcumin (CuR), glucuronide (COG) and curcumin sulfate (COS). On the other hand, enhancement of curcumin anticancer activity was discovered using laser irradiation in unique technology. UV-Vis absorption spectroscopy showed structure stability for laser irradiated CNP. Cytotoxicity and cell viability were measured using Sulphorhodamine-B (SRB) test. Antitumor activity of curcumin screened by measurement of survival fraction SF% of lung cancer cell line (A549) in vitro using Elisa method. CNP concentration was in range of (2.5 - 20 µg/ml). Concentration of curcumin nanocapsules inhibits proliferation of cells by 50% (IC50) as compared to the untreated control cells was (11.9 ug/l). samples at different curcumin concentration were irradiated using CW, DPSS laser 532 nm, 75J/cm2 at spot size 1cm2 for 15 min. IC50 measurements in the presence of laser irradiation was (8.3 ug/L) which means enhancement of cytotoxic activity by 30%. The capacity of these novel promising curcumin nanocapsules to cause apoptosis was assessed through apoptotic-related key gene (caspase3) expression using real-time RT-PCR, and b-actin as a housekeeping gene. Caspase3 folding activation increased by (4.9 folds) in the presence of CNP treated cell lines, and further increase to (7.4 folds) for CNP laser irradiated cells. This work demonstrates that curcumin nano-capsules created using a novel mechanical process combined with laser irradiation can be utilized successfully as an anticancer drug.
Keywords: Curcumin nanoparticles, cancer, A549 cell line, DPSS-laser, Caspase3, RT-PCR, cytotoxicity

1. Introduction

Cancer is one of the highest causes of death. Global cancer statistics for the year 2020, estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths, where lung cancer comes in 2nd place after breast cancer with 11.4% of new cases, whereas remain the leading cause of cancer death, with an estimated 1.8 million deaths representing 18% [1]. Current treatments depend on radiotherapy, chemotherapy and/or surgical excision. Chemical agents, target a specific pathway, which ultimately shrinks tumor size but often fails to prevent tumor recurrence [2]. Chemotherapy has adverse effects of targeting non-malignant dividing cells. This pharmacokinetics is due to poor solubility, stability, and metabolism pose different challenges of toxicity, inefficacy and limited bio-distribution [3].

On other hand cancers not responding to chemotherapeutic treatment are termed as drug-resistant cancers. It is complex process where different mechanisms can take place; drug efflux, suppression of apoptosis, alteration of drug metabolism, amplification of oncogene and epigenetic factors [4]. Curcumin is a pharmacologically active compound and derived from a spice called turmeric. Turmeric composed mainly of Curcumin 77%, Demethoxy curcumin 18%, and Bisdemethoxy curcumin 5%. Therapeutic activity of curcumin is due its action as effective modulator of multiple molecular targets in various signaling cascades; suppresses transformation, proliferation, inhibits metastasis of tumors and induces apoptosis through the regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes. In 1949 it was 1st time to record the anti-

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bacterial activity of curcumin. More than 20 yrs. Later therapeutic effects like antidiabetic, anti-inflammatory, anti-oxidant, anti-cancer recorded [5].

Studies shows that Curcumin can prevent a wide diversity of cancers in mice, comprising breast adenocarcinoma, forestomach, duodenal, and colon cancer, as well as skin tumors caused by 12-O-tetradecanoyl-13-phorbol ester (TPA) [6]. Curcumin's capacity to cause apoptosis in a diversity of cancer cells suggests that it may be developed as a universal cancer preventive agent. The intracellular caspases, a family of structurally similar cysteine proteases, are one of the keys signaling mechanisms involved in apoptotic cell death [7]. Caspase activity is involved in the cleavage of cellular proteins, which are often proteolyzed during apoptosis. Caspases -2, -3, -6, -7, and -9, for example, are capable of cleaving poly (ADP ribose) polymerase (PARP) [8].

Photothermal treatment (PTT) uses photosensitizing chemicals that are absorbed by cells and used to generate heat when exposed to near-infrared (NIR) light, resulting in photoablation of cancer cells and eventual cell death [9]. PTT has recently generated considerable attention for these reasons: (1) PTT is a slightly invasive cancer therapy option, as the PTT effect, which comprises light-absorber accumulation and limited laser exposure, occurs exclusively at the tumor location, deprived of causing damage to normal tissues. (2) Different from chemotherapy or molecular targeting treatment, PTT destroys cancer cells without generating resistance, independent of genetic background, and hence may be used on all cancer patients [10]. To maximize PTT efficiency and minimize nonspecific heating of healthy cells, photosensitizers should have high absorption in the near-infrared area [11]. Nanomaterial-based PTT has been widely studied in vitro and in vivo as a non-invasive or slightly invasive and effective therapeutic tactic for the treatment of numerous types of cancers [12].

Lung cancer is one of those high drug resistant cancers. Human lung cancer cell line A549 showed 50% cell viability at a high dose of 10,000 U of interferon (IFN)-alpha (IFNα) [4]. Upon pretreatment with curcumin prior to interferon studies have shown increased vulnerability of cells towards the cytotoxic activity of IFNα. It was measured as dose dependent decrease of nuclear subunits p50 (NF-κB1) and p65 (RelA) which are implicated in process of cell proliferation and differentiation, inflammatory and immune response, cell survival and apoptosis, cellular stress reactions and tumorigenesis [4].

The main reason that curcumin was not widely accepted as an ideal pharmacologically effective molecule its limitations associated with its physicochemical parameters such as its poor solubility and bioavailability. Nano-medicine is a promising tool to obtain effective drug delivery. Since nano-based drug provide enhanced transport across different biological barriers which enables selective targeting of malignant cells specifically. It can overcome problems like stability, solubility, drug degradation from cell enzymes. Thus, increasing half-life of the drug in systemic circulation; improves drug distribution and targeting cancer sites.

2. Material and Methods

2.1. Curcumin nanoparticles (CNP)

Curcumin nanoparticles was prepared from turmeric powder by mechanical technique using Ball milling (Model: PQ-N2 Planetary Ball Mill, Gear Drive 4- station – planetary Ball mill, 220 v) to increasing the ability of extraction from traditional methods. Grinding process carried out using equal amounts of stainless-steel balls ranging from 0.5-1.5 cm in diameter of total number 250, with the weight ratio of ball: powder (10: 1). Samples were grinded for different time intervals of 30, 60 and 90 min., at 40,000 rpm. as been mentioned [13]. This enhances solubility especially for drugs with limited aqueous solubility. The products are filtered by a percentage to remove impurities and separate the balls.

2.2. Curcumin Nano-encapsulation

The preparation was carried out using a modified technique of homogenization (Homogenizer PRO 400 PC, Germany) in an Alginate and Tween 20 (T20) matrix [13]. Curcumin extract was used in the following proportions; extract: Alg: T20 (3:10:1), resulting in an optimal yield of powder with desired properties [14].

2.3. Curcumin characterization

2.3.1. HPLC-Mass spectroscopy

Three-fold XEVO TQD quadruple instrument (Water Corporation, Milford, MA01757 USA mass spectrometer) was used to acquire ESI-MS positive and negative ion mode. ACQUITY UPLC-BEH reverse phase C-18 column, 1.7 µm particle size -2.1 × 50 mm, and mobile phase elution flow rate 0.2 mL/min. Elution was performed using solvent system consisted of (eluent A) Water acidified with 0.1 % formic acid & (eluent B) Methanol acidified with 0.1 % formic acid in gradient mobile phase. Control and nano-curcumin powder were subjected to LC-ESI-MS analysis. Injection of ten micro-litter samples was done into the UPLC instrument. Mass

spectra were detected as well in the ESI between m/z 100–1000. Mass spectrum with reported data and processed using the Maslynx 4.1 software was used.

2.3.2. Absorption spectroscopy

Absorption spectra of curcumin nanoparticles dissolved in methanol measured before and after irradiation with diode laser at 532nm for 15 min

2.3.3. Transmission Electron Microscopy (TEM)

Curcumin nanocapsules were suspended and characterized morphologically using pipettes and grids (carbon-coated 400 mesh copper grids) on specimens [15] using (JEOL JEM-1400 Electron Microscope).

2.4. Laser irradiation source

Laser source was continuous-wave (CW) Low-power diode laser (DPSS laser-LSR-PS-1, Lasever Inc., China) 532nm, 80 mW, gives 75 J/cm2 at spot size 1cm2. Curcumin nanocapsules added to cell lines (A-5489) was irradiated for 15 min before incubation for 24 hrs.

2.5. Biological assessments

2.5.1. Cytotoxicity assay

Cytotoxicity of was determined using the Skehan et al. assay [16]. Epithelial cell lines from human lung adenocarcinoma (A549) Cells were plated on a 96-multiwell plate (104 cells/well) for 24 hours to allow cells to adhere to the plate wall. Series of curcumin nanoparticles concentrations (2.5, 5.10, 20 µg/ml) was prepared. Each concentration dosage, were applied to the cell monolayer triplicate wells for 24 hours at 37°C and 5% CO_2, monolayer cells were cultured. Cells were fixed, washed, and stained with Sulfo-Rhodamine-B stain. Acetic acid used to eliminate the excess stain, and Tris EDTA buffer to recover the attached stain. The intensity of the colors was determined using an ELISA reader at 570 nm. The relation between surviving fraction and drug concentrations is plotted to get the survival curve of tumor cell line. The percentage of cell survival was calculated as follows: Survival fraction= O.D (Treated cells) /O. D (control cells). Same experiment was repeated where multiwell plate irradiated with laser 532nm, 75 J/cm2 for 15 min prior to incubation

2.5.2. Assessment of caspase3 expression using RT-PCR

Real-time PCR assay was used to determine the mRNA levels of relative apoptotic gene expressions. Total RNA was isolated from NCI-H460 cells after 24 and 48 hours of treatment with 25 µM curcumin using Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA, the USA), as previously reported by Chung JG [17]. RNA samples were reverse-transcribed using the supplier's standard technique (Applied Biosystems, Foster City, CA, the USA). Two SYBR Green PCR Master Mix (Applied Biosystems) and 200 nM of forward (F) and reverse (R) primers were used for quantitative PCR as shown in (Table 1).

Table 1: Primers Used for quantitative real-time PCR Analysis

Gene	Primer sequence
Caspas3	F: 5'- TTTGTTTGTGTGCTTCTGAGCC-3' R: 5'- ATTCTGTTGCCACCTTTCGG -3'
β-actin	F: 5'-CTGTCTGGCGGCACCACCAT-3' R: 5'-GCAACTAAGTCATAGTCCGC -3'

Each test was completed in triplicate on an Applied Biosystems 7300 Real-Time PCR, and the comparative CT technique was used to determine the expression fold-changes [18]. The data were examined statistically using SPSS software and the Waller Duncan ratio [19].

3. Result and discussion

3.1. Characterization of Curcumin nanoparticles

The morphology of the prepared curcumin nanoparticles was evaluated using transmission electron microscope (TEM). The TEM image of curcumin nanoparticles was shown in (Figure 1). It shows spherical micelles nanocapsules with smooth surface between 7 and 42 nm in diameter.

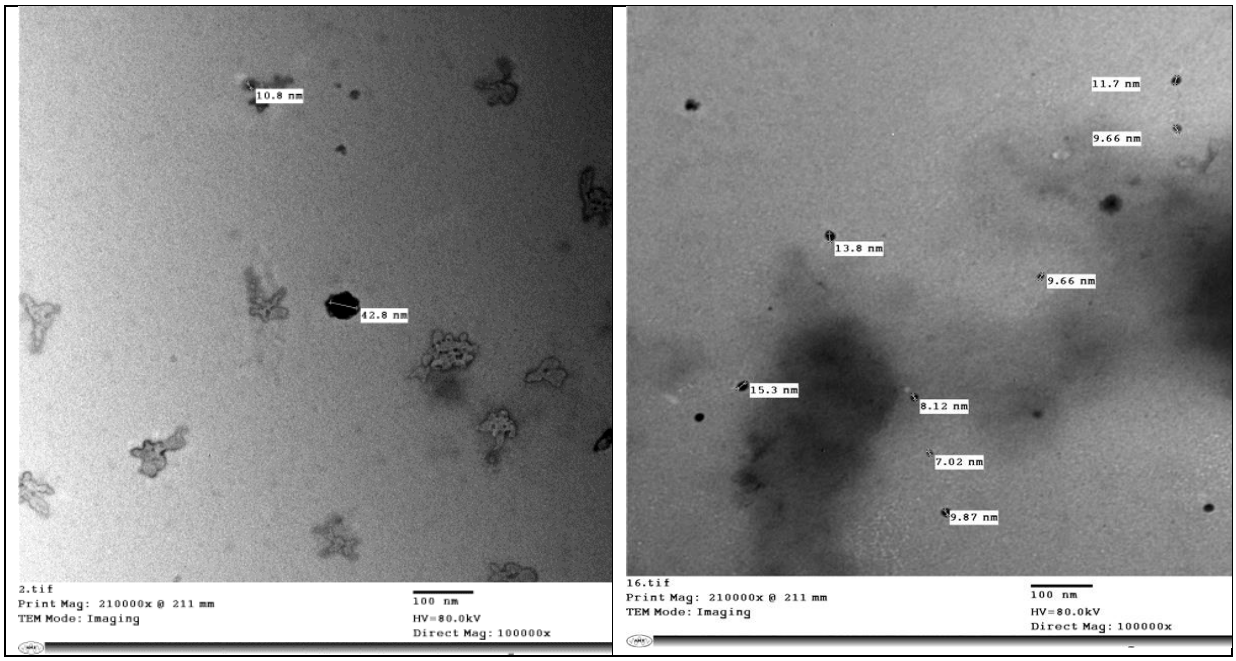


Figure 1: Transmission electron microscopy of curcumin nanocapsules

Furthermore, the absorption spectrum for curcumin nanoparticles (CNP) was dispersed in methanol as revealed in (Figure 2). It gives absorbance between 424-435 nm with λ_{max} at 420 nm. In spite of instability of curcumin [20] curcumin nanocapsules (CNP) irradiated with diode laser at 532nm for 15 min shows same λ_{max} indicates chemical structure stability. Correlated study [21] of physicochemical characterization of encapsulated curcumin in ZEIN-HTCC complexes found absorption stability within (3-12h) of natural light, indicating that the light stability of curcumin nanoparticles can be greatly improved after the encapsulation. After heat treatment as well, the curcumin capsulation exhibited remarkable high thermal stability.

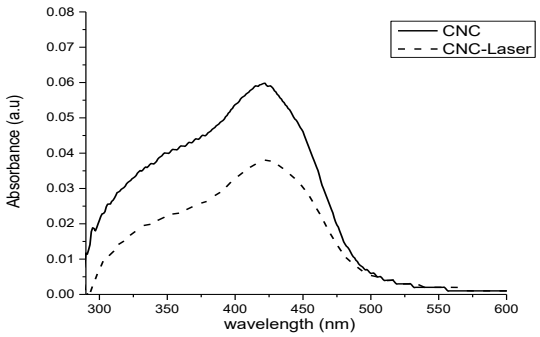
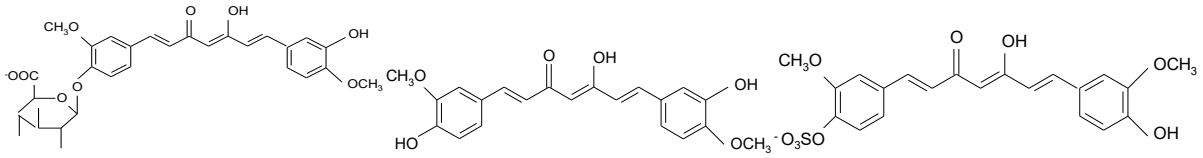


Figure 2: Absorbance spectra of curcumin nanoparticles capsulated (CNC) before and after laser irradiation

HPLC-MS measurement of curcumin nanoparticles (CUR-B), and its metabolites has been determined using ESI-MS positive and negative ion acquisition mode for bulk and nanoform. Mass spectroscopy has identified three main components 16, 20 and 21 minutes by area percentages 41, 16 and 9% in control, respectively; curcumin glucuronid (COG-A) and curcumin sulfate (COS-C) (**Figure 3**).



A B c

Figure 3: structure of curcumin (CUR-B), curcumin glucuronid (COG-A) and curcumin sulfate (COS-C).

These area percentages for the main compounds were decreased from the control to the grinded sample at 90 min using ball milling (**Table 2**).

Table 2: mass area percent for identified curcumin compounds in bulk and nanoform

Identified compound	Area % bulk	Area % nanoparticles
CUR	41	37
COG	16	14
COS	9	8

The dominant compound with MW 367, and fragmentation as follows; m/z 337 and 307, was identified as curcumin. That was in agreement with Ma *et al*. [22] who identified curcumin using LC-MS/MS. Curcumin glucuronid (COG) with MW 543 was the second compound with mass fragments as m/z 293 and 250. Finally, MW 448 curcumin derivative known as curcumin sulfate (COS) has been found showed mass fragments as m/z 125, and 219. Hardly find a significant difference between derivative peaks in both control and grinded samples. Similar results have been showed in the identified compounds from curcumin extracts [23].

3.2. Cytotoxicity Assay

Antitumor activity of new synthesized curcumin nano-capsules evaluated through cytotoxicity assay for lung cancer cell line (A549) in vitro by Elisa method. Survival curve (**Figure 4**) showing cell survival fraction (SF%) against curcumin nanocapsules in concentration range of (2.5- 20 µg/ml) in the presence and absence of laser irradiation.

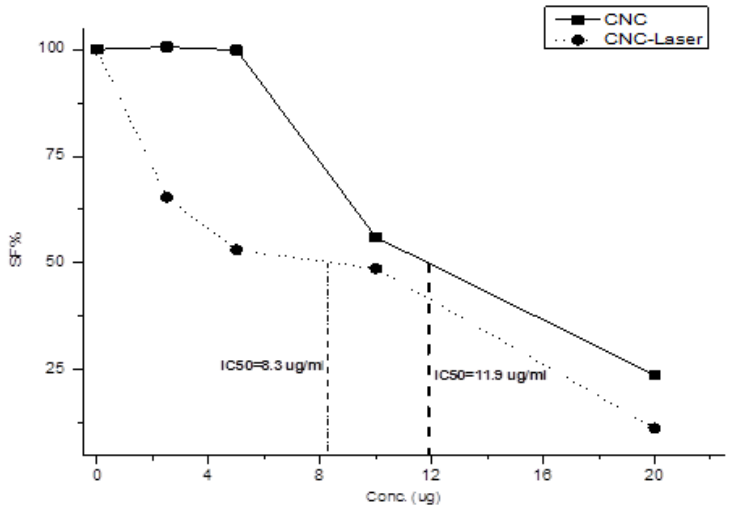


Figure 4: concentration survival curve for curcumin nano-capsule on A549 cell line growth, in the presence and absence of laser irradiation

Cytotoxic activity expressed as IC50; concentration of the curcumin nano-capsules in ug/mL inhibits proliferation of the cells by 50% as compared to the untreated control cells. Cell treated with curcumin nanocapsules shows IC_{50} value of 11.9 µg/ml, while combining nanocapsules with laser irradiation IC_{50} dropped to 8.3 µg/ml (**Table 3**). This means enhancement of cytotoxic activity by 30% by laser. This might be through oxidation and production of reactive oxygen species (ROS) [24].

Table 3: cytotoxicity assay for CNC concentration against non-small cell lung cancer (A549) in the presence and absence of laser irradiation

sample	CNC			CNC -Laser		
Conc µg	***S.F%***	***Error***	***I.G%***	***S.F%***	***Error***	***I.G%***
0	100	0.05	0	100	0.05	**0**
2.5	100.6	0.050	0.59	65.4	0.033	**34.6**
5	99.85	0.049	0.15	53.1	0.027	**46.9**
10	56.05	0.028	44	48.7	0.024	**51.3**
20	23.78	0.011	76.2	11.3	0.006	**88.7**
IC50	***11.9 µg***			***8.3 µg***		

3.3. Caspase3 gene expression

The impact of curcumin nanocapsules on mRNA expression of caspase-3 and associated genes in A549 cell was determined using real-time RT-PCR. The standard curves for the housekeeping gene, β-actin, were efficiently established by determining the amplification efficiency of β-actin genes using real-time PCR. A linear regression study of standard curves revealed a strong association with β-actin. Real-time PCR analysis revealed a statistically significant difference in the fold change of mRNA expression for the apoptotic gene caspase-3 (**Figure 5**).

There was an increase in the expression of caspase3 gene for cells Preincubated with CNC by of 4.9-fold and further enhancement to 7.4-fold in case of cell lines subjected to laser irradiation for 15 min in presence of CNC prior to incubation. Given the critical function of caspase-3 in apoptosis, results demonstrated that combination of laser irradiation with nanomedicine CNC enhance apoptosis in lung cancer cell lines and that curcumin's growth inhibitory effects appear to be mediated through caspase-3 gene regulation. These results are consistent with other research finding where Curcumin inhibits development of lung cancer NCI-H460 cells by inducing apoptosis or cell cycle arrest and activating caspase-3 [25]. Recently Mirakabad et al. record that combination of curcumin dose-dependently of 5 μM and laser has a significant neuroprotective effect on cells where a decrease in Bax/Bcl2 ratio was an indicator of apoptosis [26]. Curcumin treatment of the non-small-cell lung cancer (NSCLC) cell lines A427 and A549 enhanced the microRNA miR-192-5p and reduced cell growth and migration via the Wnt/-catenin pathway [27].

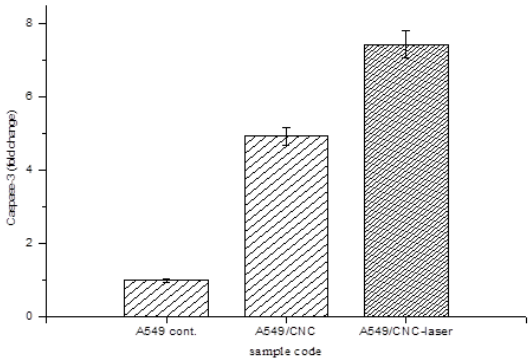


Figure 5: Caspase 3 gene fold change for A549 cell line control, incubated with curcumin nanoparticles in presence and absence of laser irradiation

Micelles and nanoparticles have been employed to efficiently penetrate and release curcumin. When treated with polymeric micelles containing doxorubicin and curcumin, the A549/ADR lung cancer cell line demonstrated doxorubicin resistance reversal [28, 29]. In our study Curcumin nanocapsules (CNC) as anticancer have shown enhanced activity by 532 nm green diode laser. Different Photochemical mechanisms of light-triggered release from nanocarriers have been reported [30]. These mechanisms including photoisomerization, photo crosslinking, and photosensitization-induced oxidation, Surface plasmon absorption by gold nanoparticles and photothermal effects, photochemical hydrophobicity switch, Polymer backbone photo-degradation, Photo de-crosslinking. NIR light-triggered release of hydrophobic drug curcumin encapsulated in Ag/Au bimetallic nanocarriers with 70% release of curcumin was achieved upon irradiation at 37oC, same results were achieved without irradiation by rising the incubation temperature to 41oC confirm that the stimulated release is due to photothermal sensitivity [31].

Visible light sensitive–titanium dioxide nanoforms for codelivery of Fe2+-dependent drug artemisinin showed improved antitumor effects through synchronized drug tumor-responsive release, and generated ROS both in vitro and in vivo [32]. In Minimal Disease Residual cells, nano medicine or nano carrier-based techniques show potential. Nanotechnology enhances the bioavailability and transfer of functional characteristics across cell membranes. The use of nano-formulations derived from phytochemicals enhances treatment response and targets drug resistance. Combination treatment with phytochemicals and chemotherapeutics has a bright future [33].

4. Conclusion

The present study evaluates the activity of curcumin nano-capsules (CNC) synthesized using a novel mechanical process as anticancer drug. Cytotoxicity measurement determined the ability of CNC to inhibit proliferation of lung cancer cells A549. Promising results for one of the most drug resistance cancer cells were determined. Visible light laser irradiation combined treatment with nanoparticles shows further improvement of cytotoxic activity by 30%. Molecular processes behind static action analyzed through apoptotic-related genes suggest

key gene (caspase3) to have a major role in the process. This also confirmed by increase in caspase 3 activity after cells was subjected to laser irradiation. These results suggest further in-vivo experiments for combined protocol of CNC and laser as a reliable anticancer drug delivery system.

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6. References

[1]. Sung, H, Ferlay, J, Siegel, RL, Laversanne, M, Soerjomataram, I, Jemal, A, Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021, 71, 209- 249.

[2]. Yallapu MM, Jaggi M, Chauhan SC. Curcumin nanoformulations: a future nanomedicine for cancer. Drug Discov Today. 2012, 17, 71-80.

[3]. Navya, P.N., Kaphle, A., Srinivas, S.P. et al. Current trends and challenges in cancer management and therapy using designer nanomaterials. Nano Convergence, 2019, 6, 23

[4]. Shaikh S, Shaikh J, Naba YS, Doke K, Ahmed K, Yusufi M. Curcumin: reclaiming the lost ground against cancer resistance. Cancer Drug Resist. 2021, 4, 298-320.

[5]. Fiona C. Rodrigues, N.V. Anil Kumar, Goutam Thakur, Developments in the anticancer activity of structurally modified curcumin: An up-to-date review, European Journal of Medicinal Chemistry, 2019, 177, 76-104

[6]. Verma S.P., Salamone E., Goldin B., Curcumin, and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides, Biochem. Biophys. Res. Commun. 1997, 233, 692–696.

[7]. Salvesen G.S., Dixit V.M., Caspases: intracellular signaling by proteolysis, Cell. 1997, 91, 443–446.

[8]. Nagata S., Apoptosis by death factor, Cell. 1997, 88, 355–365.

[9]. Shibu E. S., Hamada M., Murase N. and Biju V., Nanomaterials formulations for photothermal and photodynamic therapy of cancer. Photochem J., Photobiol., C, 2013, 15, 53.

[10]. Tsuruo T., Naito M., Tomida A., Fujita N., Mashima T., Sakamoto H. and Haga N., Cancer Sci., Molecular targeting therapy of cancer: drug resistance, apoptosis and survival signal. 2003, 94, 15.

[11]. Camerin M., Jori G., Della Ciana L., Fabbroni S., Bonacchi S., Montalti M. and Prodi L., Photothermal sensitisation and therapeutic properties of a novel far-red absorbing cyanine. Photochem. Photobiol. Sci. 2009, 8, 1422.

[12]. Yang K., Zhang S., Zhang G., Sun X., Lee S.T., Liu Z., Graphene in mice: ultrahigh in vivo tumor uptake and efficient photothermal therapy. Nano Lett. 2010, 10, 3318–23.

[13]. Loh, Z. H., A. K. Samanta and P. W. S. Heng. Overview of milling techniques for improving the solubility of poorly water-soluble drugs." Asian journal of pharmaceutical sciences. 2015, 10, 4, 255-274

[14]. Terjung N., Löffler M., M. Gibis, Hinrichs J. and Weiss J., "Influence of droplet size on the efficacy of oil-in-water emulsions loaded with phenolic antimicrobials. Food & function. 2012, 3, 290-301.

[15]. Abd-Elsalam K. A. and Khokhlov A. R., "Eugenol oil nanoemulsion: antifungal activity against Fusarium oxysporum f. sp. vasinfectum and phytotoxicity on cottonseeds." Applied Nanoscience. 2015, 5, 2, 255-265.

[16]. Skehan R., Storeng R., Scudiero D., Monks A., Mahon J.M., Vistica D., Warren J.T., Bokesch H., Kenney S., Boyd M.R. New colorimetric cytotoxicity assay for anticancer-drug screening. Natl. J. Cancer Inst. 1990, 82, 1107–1112.

[17]. Chung J.G., Yeh K.T., Wu S.L., Hsu N.Y., Chen G.W., Yeh Y.W. and H.o., HC: Novel transmembrane GTPase of non-small cell lung cancer identified by mRNA differential display. Cancer Res, 2001, 61, 8873-8879.

[18]. Pillai G.R., Srivastava A.S., Hassanein T., Chauhan D.P., Carrier E., Induction of apoptosis in human lung cancer cells by curcumin Cancer Letters. 2004, 208, 163–170.

[19]. Waller R.A., & Duncan D.B.A., Bayes rule for the symmetric multiple comparisons problem. Journal of the American Statistical Association. 1969, 64, 1484-1503.

[20]. Chuacharoen, T. and C. M. Sabliov. Comparative effects of curcumin when delivered in a nanoemulsion or nanoparticle form for food applications: Study on stability and lipid oxidation inhibition. LWT-Food Science and Technology: 2019, 108319.

[21]. Ren, G., He, Y., Liu, Ch., Ni, F., Luo, X., Shi, J., Song, Y., Li, T., Huang, M., Shen, Q. and Xie, H. Encapsulation of curcumin in ZEIN-HTCC complexes: Physicochemical characterization, in vitro sustained release behavior and encapsulation mechanism. LWT Food Sci. Technol. 2022, 155, 112909

[22]. Kunati, S. R., S. Yang, B. M. William and Y. Xu. An LC–MS/MS method for simultaneous determination of curcumin, curcumin glucuronide and curcumin sulfate in a phase II clinical trial." Journal of pharmaceutical and biomedical analysis. 2018, 156, 189-198.

[23]. Ma, W., J. Wang, Q. Guo and P. Tu. "Simultaneous determination of doxorubicin and curcumin in rat plasma by LC–MS/MS and its application to pharmacokinetic study." Journal of pharmaceutical and biomedical analysis. 2015, 111, 215-221.

[24]. Shaker, E., Fawzy, M., Mahmoud, Kh., Moawad, M. and El Shafei, M. (2022) Nanocapsulation of curcumin and its protective effects against oxidative stress and carcinoma HepG2, MCF7 cells. Egypt. J. Chemistry (In press)

[25]. Wu S.H., Hang L.W., Yang J.S., Chen H.Y., Lin H.Y., Chiang J.H., Lu C.C., Yang JL., Lai T.Y., Ko Y.C., Chung J.G., Curcumin induces apoptosis in human non-small cell lung cancer NCI-H460 cells through ER stress and caspase cascade- and mitochondria-dependent pathways. Anticancer Res. 2010, 30, 6, 2125-33.

[26]. Mirakabad T.F.S., Khoramgah M.S., Tahmasebinia F., Darabi S., Abbaszadeh H.A., Khoshsirat S., The effect of low-level laser therapy and curcumin on the expression of LC3, ATG10, and BAX/BCL2 ratio in PC12 cells induced by 6-hydroxide dopamine. J Lasers Med Sci. 2020, 11, 3, 299-304.

[27]. Pan Y., Sun Y., Liu Z., Zhang C., miR-192-5p upregulation mediates the suppression of curcumin in human NSCLC cell proliferation, migration, and invasion by targeting c-Myc and inactivating the Wnt/β-catenin signaling pathway. Mol. Med. Rep. 2020, 22, 1594-604.

[28]. Gu Y., Li J., Li Y., et al., Nanomicelles loaded with doxorubicin and curcumin for alleviating multidrug resistance in lung cancer. Int J Nanomedicine. 2016,11, 5757-70.

[29]. Shaikh S., Shaikh J., Naba Y.S., Doke K., Ahmed K., Yusufi M., Curcumin: reclaiming the lost ground against cancer resistance. Cancer Drug Resist. 2021, 4, 298-320.

[30]. Nadezda F., Jagadis S., Adah A.,Photochemical mechanisms of light-triggered release from nanocarriers,Advanced Drug Delivery Reviews, 2012, 64, 11, 1005-1020

[31]. Wu W, Shen J, Banerjee P, Zhou S. Water-dispersible multifunctional hybrid nanogels for combined curcumin and photothermal therapy. Biomaterials. 2011, 32, 598-609

[32]. Zhang H., Zhang H., Zhu X., Zhang X., Chen Q., Chen J., Hou L., Zhang Z. Visible-light-sensitive titanium dioxide nanoplatform for tumor-responsive Fe2+ liberating and artemisinin delivery. Oncotarget. 2017, 8, 58738-58753.

[33]. More M.P., Pardeshi S.R., Pardeshi C.V., Sonawane G.A., Shinde M.N., Deshmukh P.K., Naik J.B., Kulkarni A.D., Recent advances in phytochemical-based Nano-formulation for drug-resistant Cancer, Medicine in Drug Discovery. 2021, 10,100082