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Cytotoxic effect of zinc oxide nanoparticle and diode laser combination in colorectal cancer in vitro via induction of cell cycle arrest and apoptosis

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ABSTRACT

The fourth deadliest reason for cancer-related deaths worldwide was found to be colorectal cancer. Zinc oxide nanoparticles (ZnO NPs) have been researched for biomedical applications due to their demonstrated anticancer, antidiabetic, antibacterial, and anti-inflammatory activities. The cytotoxic reactive oxygen species (ROS) that are produced when photosensitizers (PSs) are activated by light, which in turn cause cancer cell death, and photodynamic treatment (PDT) causes selective cytotoxicity to malignant cells. The cytotoxic effect of different concentrations of nanoparticles and laser wavelengths was studied on colorectal cancer cell line (CaCo2), while the use of combination therapy had clear effects on early apoptosis. The half-maximal inhibitory concentration (IC50) of nanoparticles for the cells was evaluated. The proper IC50 for CaCo2 cells was 20 ug/ml Zinc oxide nanoparticle and Diode laser power is 5 j/cm2. The percentage of cells that decreased in the G2 phase of the cell cycle relative to the S and G1 phases was significantly different when the combination of laser and zinc nanoparticles was used. Combined use of a diode laser and ZnO_2 significantly inhibited the spread of cancer by boosting the expression of the p53 gene. Bax, a central cell death regulator, was expressed significantly in combined treatments. However, combined use of the laser and ZnO_2 did not reveal any substantial differences in the Bcl2 gene. In conclusion, this study suggests that combination therapy was cytotoxic to CaCo2 cells in vitro and there is a possibility of developing it as an effective therapeutic agent.

INTRODUCTION

Colorectal cancer is the fourth most widespread reason for cancer-related death, accounting for 9.2 % globally, in women, it is the second-most common adult cancer, and in men, it is the third-most common adult cancer [1, 2]. Around 900,000 fatal colorectal cancer cases were reported in 2018, and by 2035, it's believed that there would be approximately 2.5 million additional cases [2, 3]. The proximal colon is mostly affected by around 41% of all colorectal cancers, whereas the distal colon and the rectum are each affected by 22% and 28%, respectively [4]. Men and women with colon cancer are on average 68 and 72 years old, respectively at diagnosis, whereas both sexes with rectal cancer are on average 63 years old [5]. The development of colorectal cancer is significantly influenced by genetic, environmental, and aging factors [6]. Chemotherapy, immunotherapy, radiation, therapy, surgery, targeted therapy, and hormone therapy are some of the traditional therapeutic modalities utilized in the treatment of cancer [7, 8]. With their capacity for cytostasis and cytotoxicity, chemotherapy and radiation treatment are frequently associated with a significant risk of recurrence and severe side effects [9]. Neuropathies, bone marrow suppression, hair loss, gastrointestinal and skin conditions, and exhaustion are among the most often seen side effects. In addition, a few adverse effects of certain drugs exist, such as

pulmonary and cardiotoxicity caused by anthracyclines and bleomycin [10]. Recent research showed that in addition to medications, in the diagnosis and treatment of cancers, genes, imaging agents (with diagnostic capabilities), and nanoparticles (NPs) provide several advantages [11]. In the past twenty years, the properties of NPs have allowed for the development of several NP-based therapeutic options [12]. ZnO NPs may specifically enter tumor cells, interact with malignant cells, and kill them, according to research done on human lung epithelial cells (L-132) and cell line A549 from human alveolar adenocarcinoma [13-15]. Photodynamic therapy (PDT), compared to other prevalent cancer therapies like radiation and chemotherapy, induces acute inflammation in addition to directly killing cancer cells, which stimulates the immune system and enhances T-cell exposure to tumor-derived antigens [16]. PDT is a well-known and successful approach for the treatment of several cancer types, including gastrointestinal (GI) cancer [17-19].

The goal of the study is to provide insights into the potential use of this combination therapy as a more effective and targeted approach to treating colorectal cancer. Testing the expression of some apoptotic genes in colorectal cancer cells with and without treatment of combined therapy.

MATERIALS AND METHODS

Preparation of cell lines and cell cultures

The National Cell Bank of Iran (NCBI) provided the colorectal cancer cell line (CaCo2). In Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA), the cells were grown with 10% heat-inactivated fetal bovine serum (FBS, Gibco, USA), 1% L-glutamine, and 1% antibiotics (penicillin/streptomycin), and incubated at 37°C in a humid environment (5% CO_2). All other chemicals were purchased from Gibco, USA, otherwise stated.

Zinc oxide nanoparticle synthesis

Zinc nitrate (0.5 M) was slowly added to a sodium hydroxide solution (1 M), and the mixture was constantly agitated for 15 minutes. Centrifuging and repeatedly washing with distilled water was done for the resulting white precipitate. 60°C hot air oven was used for drying with the white precipitate powder ($Zn(OH)_2$). Finally, $Zn(OH)_2$ transformed into ZnO after the drying process.

Laser therapy

CaCo2 cells were treated with different concentrations of nanoparticle (0.5-1000 ug/ml). Then, three concentrations of nanoparticle (10, 20 and 40 ug/ml) were chosen for treatment with Medical Diode Laser (IMDSL, India). Three laser power were tested with three concentrations such as 3 j/cm 2 (0.2W and 15 s), 5 j/cm 2 (0.5W and 10 s), and 6.4 j/cm 2 (0.8W and 8 s). Finally, the proper IC50 for ZnO NPs was 20 ug/ml and diode laser power 5 j/cm^2 were used for subsequent study with the for the cells.

Cell viability

In CaCo2 cells, 96-well plate was used to perform the MTT assay (MTT kit Merck, Germany), which measures the cytotoxicity of ZnO NPs by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide [20]. In the appropriate media,

CaCo2 cells were grown to around 70% confluence and then treated with various doses (0.5, 1, 5, 10, 20, 40, 75, 100, 200, and 1000 µg/ ml) of ZnO NPs for 24, 48, and 72 h at 37 °C. After the first incubation period, the plate was incubated for an additional 4 h with MTT solution (5 mg/mL in the medium, Sigma-Aldrich, USA). DMSO (100ml/well, Sigma-Aldrich) was used to replace the supernatant, and the absorbance was measured at 540 nm. Three times each of the trials were run in duplicates. The sample concentration known as IC50 (half-maximal inhibitory concentration) inhibits 50% of the cells.

Propidium iodide staining for determining cell cycle

Six-well plates with $5x10^5$ cells per well were used to seed the cells, which were then subjected to the ZnO NPs and laser treatments. In polypropylene flow cytometry tubes, 1.2 mL of ice-cold DPBS was used to resuspend the cells after they had been washed twice with DPBS. After being kept at -20°C for the overnight, the fixed cells were twice washed by centrifuging at 200x g for 10 min at 4°C and aspirated the supernatant. After that, 2.8 mL of ice-cold 100% ethanol was added. After being resuspended in a newly produced propidium iodide (PI) staining solution for 15 min, cells were incubated at 37°C while being sheltered from light. The solution contained 200 g/mL PI, 200 µg/mL RNase A, and 0.1 percent (v/v) Triton X-100. Evaluation of the stained cell was carried out using a FACSCanto II flow cytometer (BD Biosciences). Calculation of the proportion of cells in the G0/G1, S, and G2/M stages of the cell cycle was done using the Watson (Pragmatic) model in FlowJo v10.4.1 (FlowJo, LLC, Ashland, OR, USA).

Annexin V with propidium iodide staining for detection of apoptosis

Following the manufacturer's instructions, before being stained with an Annexin-V-FLUOS labeling kit (Roche Diagnostics in Mannheim, Germany), cells were washed twice with DPBS. To get necrotic cells, heat was applied to a cell suspension in DPBS for 30 min at 63°C. A FACSCanto II (BD Biosciences) was used to examine the cells, capturing at least 10,000 single-cell events per sample while gating out debris and doublets. FlowJo v10.4.1 was used to quantify viable (double-negative), necrotic cells (PI-positive) and early apoptotic (annexin V-positive), and late apoptotic (annexin V and PI double-positive) cells (FlowJo, LLC) using FACSCanto II (BD Biosciences).

RNA extraction and cDNA synthesis

Using Trizol reagent (Invitrogen, USA), total RNA was extracted from cells as per manufacturer's instructions. UV spectrophotometry at 260 nm was used to measure the concentration of RNA. Using a Quantitect Reverse Transcription Kit and oligo (dT) primers, RNA samples were used to make cDNA (Qiagen, USA). The primers are shown in Table 1. RNA samples, RNase-free water, Quantiscript RT Buffer, Quantiscript® Reverse Transcriptase, genomic DNA Wipeout Buffer, RT Primer Mix, and Quantiscript RT Buffer at room temperature (15–25 °C) were thawed on ice. Flip the tubes to combine each solution. After the genomic DNA elimination process, centrifugation was performed briefly to collect any remaining liquid from the tubes' sidewalls, and then keep it. Incubate 2 µl of gDNA, 4 µl of RNA, and 8 µl of RNase-free water in a total volume of 14 µl for two min at 42 °C before placing the mixture immediately on ice. There should be a master mix for reverse transcription and stored on ice using the following ingredients: 1 µl of Quantiscript Reverse Transcriptase, 4 µl

of Quantiscript RT Buffer, 1 µl of RT Primer Mix, and 14 µl of the whole genomic DNA elimination procedure. The samples were incubated for 15 min at 42°C. Real-time PCR is performed after cooling the reverse transcription processes. Store reverse transcription reactions at -20°C for long-term storage. The specific primers used in PCR reactions are listed in Table and were created by the program Oligo7 for designing primers (Tehran, Iran).

Table 1. Primers for apoptotic genes.

Genes	Sequences	Temperature
BAX	F: CGAGAGGTCTTTTTCCGAGTG	61°C
	R: GTGGGCGTCCCAAAGTAGG	61°C
BCL2	F: AGCATCACGGAGGAGGTAGAC	63°C
	R: CTGGATGAGGGGGTGTCTTC	62.5°C
P53	F: CGTGTGGAGTATTTGGATGAC	59.4°C
	R: TTGTAGTGGATGGTGGTACAGTC	62°C

Quantitative reverse transcription PCR (qRT-PCR)

Using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and SYBR Green master Premix Ex Taq Kit from the Korean company (Takara Biotechnology), the expression levels of all genes were measured. The PCR process started with an initial melting cycle to activate the polymerase reaction for 5 min at 95°C, followed by 40 cycles of melting 30s at 95°C, annealing 30s at 58°C, and extension 30s at 72°C. The 10 µl amplification reaction contained cDNA, 0.2 M of each gene-specific primer, and 5 µl SYBR green master solution. As a reliable internal control gene, Beta-actin, the levels of expression were normalized and expressed as $2^{-\Delta Ct}$. Primer effectiveness for the investigated genes was compared with that of the reference genes.

Determination of ROS

CaCo2 cells were subjected to varying doses of ZnO NP treatment, followed by incubation with 10 µM of dichlorofluorescein diacetate (DCF-DA, Sigma, USA) at a temperature of 37°C for 30 min. Subsequently, the cells were washed 3 times, and their fluorescence was measured at a wavelength of 525nm, with excitation at 488nm on a microplate reader (Thermo Fisher Scientific, USA).

Statistical Analysis

The statistical analysis was carried out using the SPSS 17.0 program. Independent t-tests or one-way ANOVO TESTS were used to assess the differences in the experimental data. Information was presented as mean± standard error. Statistics were defined as significant if P values were less than 0.05.

RESULTS

Cytotoxic effect of ZnO_2 and Diode laser on CaCo2 cell line in vitro

According to the statistical analysis, the combination of laser and zinc nanoparticles significantly influenced the number of cells going through early apoptosis as a percentage compared to living, late apoptosis, and necrosis, as shown in Figure 1. The ZnO NPs and laser combination therapy has an effect on late apoptosis and is more

effective because the percentage of cells in late apoptosis decreased significantly compared to early apoptosis and necrosis (p-value<0.05). In contrast, the use of ZnO NPs and laser to reduce the percentage of cell lines had clear effects on the early apoptosis (p-value<0.05), as evidenced by the decrease in the number of cell lines in the early apoptosis, which was not evident in the late apoptosis and necrosis Figure 2. Necrosis is a distinct phase that is more resistant to all forms of treatments, and when compared to live cases, the percentage of cell lines declines by a small amount. Also, the results showed the significant effect of combination therapy on the colorectal cell line as shown in Figure 2, there are 3 experiments with different times which is shown so near to each other results.

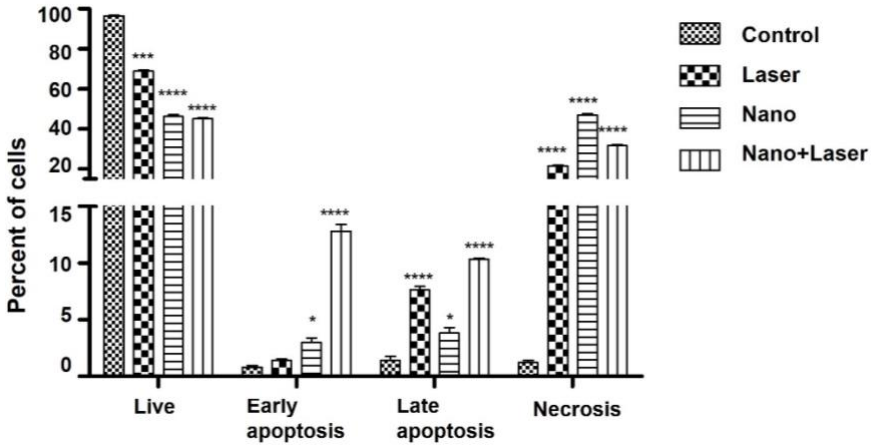

Figure 1. Effect of Zinc nanoparticles, laser, and nano-laser combinations on apoptosis in in CaCo2 cells. The dose of nanoparticle was 20 ug/ml, and laser was 5 j/cm2 (0.5W and 10 s). Cytotoxicity was measured using MTT assay. All groups compare with control group.

Effect of ZnO_2 and diode laser on cell cycle in CaCo2 cells

To investigate whether Zinc nanoparticles, Laser, and Zinc+laser affected, the regulation and analysis of the cell cycle performed in this study. Using flow cytometry, the distribution of the cell cycle phase was investigated, and PI labeling. Using Multicycle software, it was calculated how many cells were in the G1, S, and G2/M stages. All case of treatments has the same effects on the percent of cell lines in the cell's G2 phase cycles when compared to the effects on another phase of cell cycles. Figure 3 shows that when laser and zinc nanoparticles were used together, there was a big difference in terms of the proportion of cells line decreasing during the cell cycle's G2 phase compared to the S and G_1 phases (p-value<0.05). In contrast, using zinc nanoparticles and laser alone to reduce the number of cell lines undergoing various stages of the cell cycle had clear effects in different proportions (p-value<0.05), which shows that the number of cell lines decreased during G_2 by using a laser alone. Using zinc nanoparticles in the declining percent of cell lines revealed the low proportion that has a low significant difference in the decreasing when compared with the other treatment case (Figure 4).

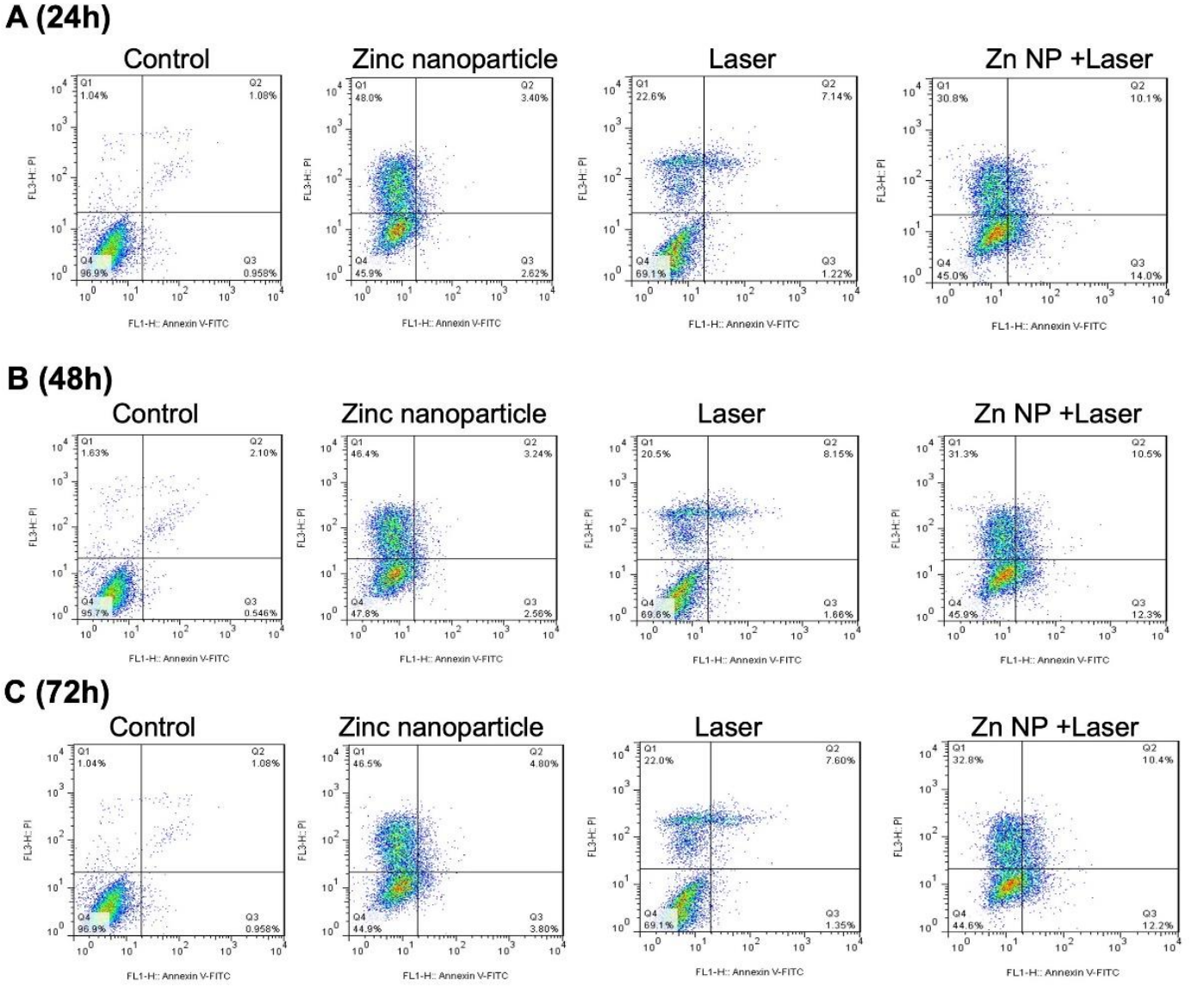

Figure 2. Effect of Zinc nanoparticles, laser, and nano-laser combinations on apoptosis and necrosis in in CaCo2 cells. Cells were treated for 24h, 48h, and 72h). *Q1: Necrosis, Q2: Late Apoptosis, Q3: Early Apoptosis, Q4: Live Cells. The dose of nanoparticle was 20 ug/ml, and laser was 5 j/cm2 (0.5W and 10 s). These were measured using flow cytometry.

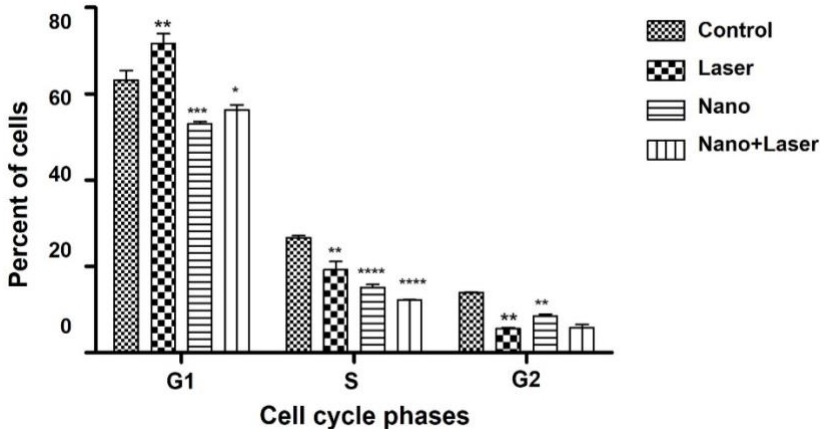

Figure 3. The graph illustrates the influence of nano, laser, and nano-laser combinations on the proportion of CaCo2 cells in different cell cycle phases. The dose of nanoparticle was 20 ug/ml, and laser was 5 j/cm2 (0.5W and 10 s). These were measured using flow cytometry. All groups compare with control group.

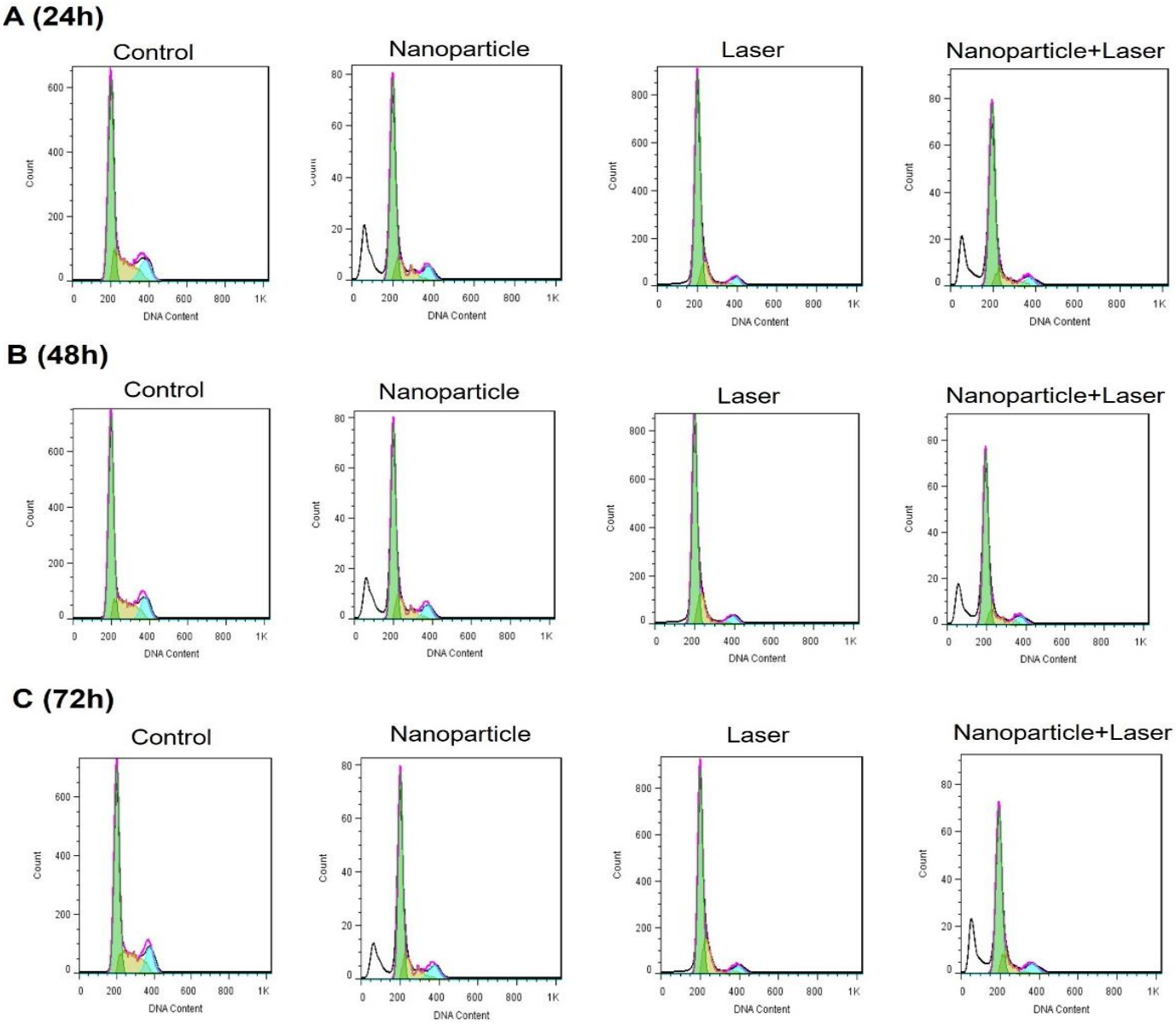

Figure 4. Effect of Zinc nanoparticles, laser, and nano-laser combinations on remaining the amount of DNA contents in CaCo2 cells with different cell cycle phases. The dose of nanoparticle was 20 ug/ml, and laser was 5 j/cm2 (0.5W and 10 s). These were measured using flow cytometry.

Effect of ZnO_2 and diode laser on ROS in CaCo2 cells

The use of zinc nanoparticles and laser alone with zinc nanoparticles and laser in combination to decrease the proportion of cell lines in DCFH- compared to DCFH+ (Figure 5), highly observed changes and strong significant differences have been recorded (p-value<0.05). the percent of cells in the control remains at high rates and decreased in all treated cases with different ratios (Figure 6). Based on the gating of living cells to check the amount of ROS. Based on the average staining ability to live cells (MFI) with fluorescent, about 90% of living cells have the staining ability, which is reported with +. But for more accuracy, we report this amount with - because 10% of living cells may not have been dyed. When DCFH is exposed to oxygen, it turns into the fluorescent molecule 2′7′-dichlorofluorescein (DCF). Dichlorodihydrofluorescein diacetate (DCFH-DA). DCFDA is good to quantify ROS and nitric oxide. But DCFH-DA is an indicator of peroxynitrite formation. Both are cell permeable and once inside the

cell, they undergo enzymatic cleavage of the diacetate groups by cytoplasmic esterase. After the removal of acetate, they readily get oxidized to form the highly fluorescent product dichlorofluorescein (DHF). DCFDA can be oxidized by H2O2, NO, or Superoxide, but DCFH-DA is oxidized by Peroxynitrite only and neither NO, superoxide, nor hydrogen peroxide alone appear to oxidize DCFH.

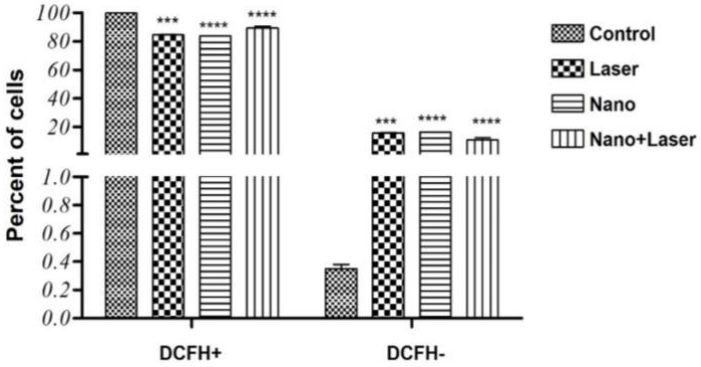

Figure 5. Effect of Zinc nanoparticles, laser, and nano-laser combinations on reactive oxygen species (ROS) using DCFH+ and DCFH- in CaCo2 cells. The dose of nanoparticle was 20 ug/ml, and laser was 5 j/cm2 (0.5W and 10 s). ROS were measured using flow cytometry. All groups compare with control group.

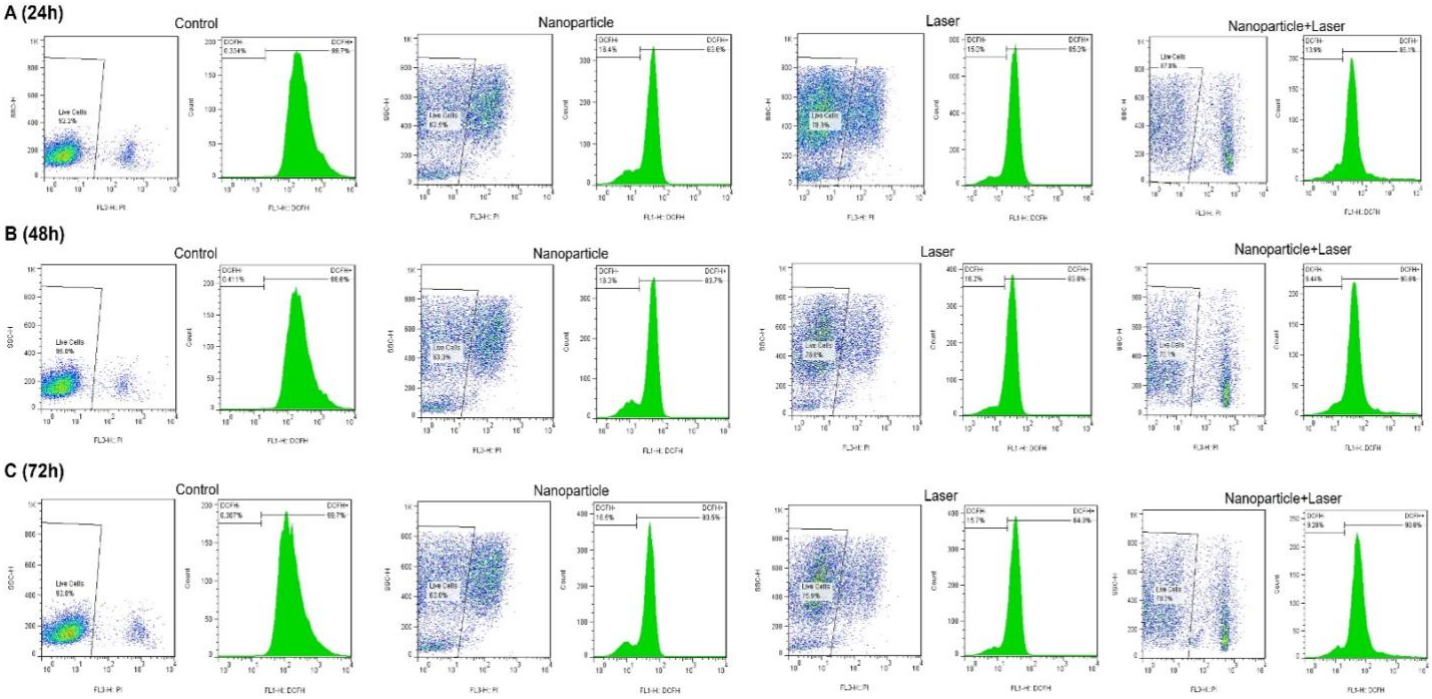

Figure 6. Effect of zinc nanoparticles, laser in alone and zinc nanoparticles with laser on the percent of CaCo2 cells in both DCFH+ and DCFH-. The dose of nanoparticle was 20 ug/ml, and laser was 5 j/cm2 (0.5W and 10 s). ROS were measured using flow cytometry. All groups compare with control group.

Effect of ZnO_2 and diode laser on expression of apoptotic genes in CaCo2 cells

Results which are investigated to examine the expression of a few apoptotic genes using Real-time PCR (P53, BAX, Bcl2) showed a highly significant difference in the use of diode laser with zinc nanoparticles to block the cancer progression by increasing the expression of the *p53* gene (p-value<0000) as shown in Figure 7. In addition, in the treatment of cancer cell lines via *BAX* gene expression (apoptosis regulator BAX), using

both nano+laser affect the expression of the BAX gene and accelerates and increases the rate of the gene and finally causes the death of cancer cell, which significantly different when compared with each of control, treating with nano and laser in alone (p-value<0.05). Nevertheless, there is no revealed significant difference in the case of the Bcl2 gene during using a combination treatment of laser with zinc nanoparticles (p-value>0.05).

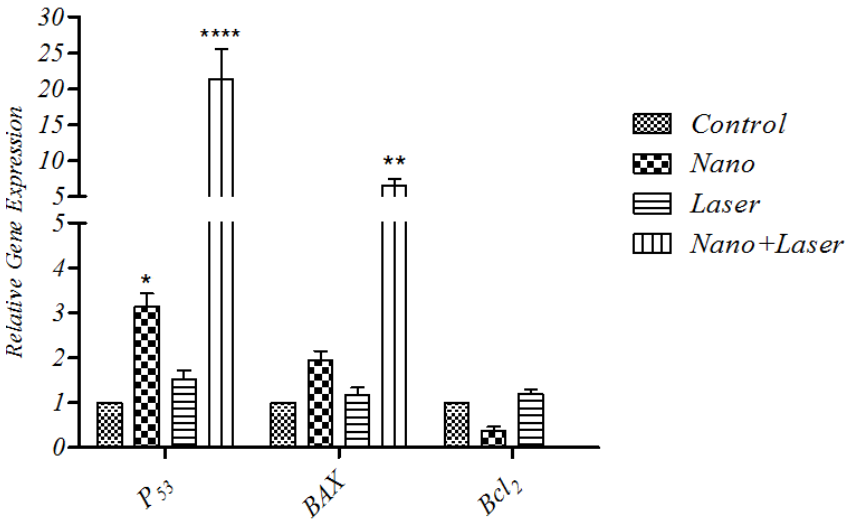

Figure 7. Graph revealed the effects of nano, laser, and nano-laser combination in the expression of apoptotic genes in in CaCo2 cells. The dose of nanoparticle was 20 ug/ml, and laser was 5 j/cm2 (0.5W and 10 s). Gene expression was measured using Real-time PCR method. All groups compare with control group.

DISCUSSION

As a heterogeneous illness, colorectal cancer progresses to the formation of polyp-like malignant tumors in the rectum and colon's inner walls [21]. The reports of WHO (2018) indicated that the second-leading cause of mortality worldwide was colorectal cancer and the third-most frequent cancer, respectively, based on the International Agency for Research on Cancer (IARC) [22, 23]. Similar to other cancer treatments such as ZnO NPs are effective in treating colon cancer [24]. In a recent study, CaCo2 cells were exposed to silver and ZnO nanoparticles to compare their cytotoxicity. It was discovered that by increasing the levels of ROS, ZnO nanoparticles are more harmful than silver NPs [25]. Also, PDT is a recognized and effective technique to treat several cancer types [17, 19].

This study used colorectal cancer cell lines through 3 different types of treatment such as ZnO NP, Laser, and ZnO NP+Laser. These therapies are used to assess the relative expression level of three different target genes including P53, Bcl2, and Bax. There is a different concentration of ZnO NPs and laser wavelength for cell cycle, ROS, and qRT-PCR. According to recent research that used laser and gold nanoparticles on colon cancer in 2020, which is indicated based NPs exhibited the most efficient cytotoxicity and markedly enhanced inhibition effect on cells proliferation to SW480 cells under laser exposure when compared to the NPs merely with PTT or chemotherapy [26]. In 2021 this study decide that Several (although small) in vitro and in vivo clinical experiments have examined the efficacy of PDT in the elimination of CRC, and the results have shown the treatment's exceptional potency with very few side effects [27]. Despite the positive results, typical PSs have limitations that prevent PDT from reaching its full potential, including inadequate tumor targeting, insufficient quantum yield, limited cellular absorption, and insufficient penetration depth [28]. In this regard,

NPs have been highlighted as favorable platforms to enhance the delivery of the PSs into the targeted CRC tumors, in 2021 [29, 30]. NPs are prospective PDT options because they might help overcome some of the challenges: they can improve the PSs' bioavailability and solubility, their tumor selectivity and specificity with minimal side effects, and their overall increased PDT efficacy [28].

Furthermore, this study show that the combination of laser and zinc nanoparticles had a significant influence on the percentage of cells undergoing early apoptosis compared to living, late apoptosis, and necrosis. ZnO NPs biologically had anticancer properties in HCT-116 cell lines, according to some research [31]. Numerous cancer cells were shown to undergo cell cycle arrest and death after being exposed to ZnO nanoparticles, according to previous studies [32, 33]. Previous data demonstrated that the viability of the treated cell lines was dose-dependent; as the concentration of ZnO NPs rose, the viability decreased [34].

Growth inhibition is frequently caused by cell cycle arrest. Propidium iodide was employed as a probe in cell cycle distribution analysis by flow cytometry to examine the effects of this substance on cell cycle progression and determine whether the action of extracts involves changes in cell cycle progression. Propidium iodide, a water-soluble dye that intercalates DNA, binds to a certain amount of DNA in a cell, and the amount of DNA in a population of cells determines where they are distributed during the cell cycle [35].

This study discovered that when laser and zinc nanoparticles were used together, compared to the S and G1 phases of the cell cycle, the percentage of cells dividing less rapidly in the G2 phase was significantly lower. According to research, ZnO nanoparticles can induce cell cycle arrest and death in a variety of cancer cells, which can inhibit the proliferation of cancer cells [32]. Cell proliferation is characterized by the progression of the cell cycle. Cancer development and progression have been connected to dysregulation of the cell cycle [36]. As a result, one of the alluring therapeutic targets for the treatment of cancer is the cell cycle. Nevertheless, cell cycle-targeting siRNA or small molecule inhibitors have been created [37]. Cell cycle arrest at the S-phase has only occasionally occurred, with the majority of chemotherapeutic drugs causing cell cycle arrest either at the G0/G1 or the G2/M stage. Cyclin-dependent kinase (CDK) enzymes are a class of proteins that regulate the cell cycle [38]. Through their connection with particular inhibitors, kinases, cyclin partners, and phosphatases, CDKs can control their activity [38].

The results demonstrate that ZnO NPs were responsible for cancer cell death by testing each therapeutic component separately and comparing them to the multi-component platform, which is consistent with other research demonstrating that ZnO NPs are highly hazardous to cancer cells. Reactive oxygen species are known to cause oxidative stress, which malignant cells produce at greater inducible levels than normal cells, which is one of the ways hypothesized to mediate toxicity [39-41].

Furthermore, our study shows that using zinc nanoparticles and laser alone with zinc nanoparticles and laser in combination to reduce the proportion of cell lines in DCFH- compared to DCFH+, highly observed changes and strong significant differences have been recorded. Earlier research has shown that for many different forms of cancer, a possible therapeutic is PDT. PDT produces ROS by utilizing interactions between a photosensitizer, light, and oxygen [42]. The amount of ROS would impact the effectiveness of PDT because ROS plays a significant role in tumor cell death by causing vascular shutdown, oxidative stress, and the immunological response [43].

The anti-oxidative capacity of cells can be depleted by high and prolonged ROS build-up, which results in cell death. Cancer cells exhibit higher amounts of cellular ROS in comparison to healthy cells due to their increased metabolic requirements and rapid rate of proliferation. Because of their innately high level of oxidative stress, cancer cells are more vulnerable to ZnO NPs therapy. Proteins may become ubiquitinated as a result of oxidative damage brought on by high ROS buildup. Proteotoxic stress results from an increase in the workload placed on the cell's protein degradation machinery by a buildup of proteins that have been ubiquitinated. In ovarian cancer cells treated with ZnO NP, we notice a decline in an anti-oxidative capacity as well as an increase in proteotoxic stress. It is known that cancer cells put together specific proteasome isoforms with higher proteolytic capacity as a result of oxidative stress brought on by metals[44].

The mitochondrial electron transport chain is thought to have a role in the creation of intracellular ROS, and it is also thought that cancer-fighting chemicals that enter cancer cells may harm the electron transport chain, which would cause a large-scale intracellular release of ROS [45]. As a result, increasing ROS levels cause mitochondrial damage, followed by apoptosis caused by an imbalance in protein activity. [46]. Because of the elevated intracellular amounts of dissolved zinc ions and increased ROS generation caused by ZnO NPs, cancer cells are cytotoxic and die through an apoptotic signaling pathway [47].

In addition to controlling the p53 tumor suppressor gene's activity, zinc plays a crucial role when the apoptosis-inducing enzyme caspase-8 is stimulated [48]. Another crucial zinc target is caspase-9 [49], Caspase-3, and other cellular death-causing enzymes that rupture nuclear membranes are powerfully induced by it. Therefore, when trying to treat cancer, Bax, p53, and caspases are regarded as important apoptotic markers. ZnO NPs were introduced to MCF-7 to assess this, and the ZnO NPs demonstrated dose-dependent suppression of cancer cells. Markers like Bax, JNK, p21, and p53 were elevated, which caused MCF-7 to undergo apoptosis [32].

Caspase-3, a crucial executioner protein in apoptosis, results in the cleavage of PARP and cell death [50]. The primary regulators of the apoptotic process are members of the Bcl-2 family proteins, which contain both pro- and anti-apoptotic proteins. Bcl-2 and Bax are pro- and anti-apoptotic proteins, respectively [51]. In the current study, PpIX-PDT boosted caspase-3, PARP, and Bax expression while lowering Bcl-2 levels. To inhibit activation of the downstream mitochondrial death cascade, Bcl-2 may bind to and sequester Bax, making the Bcl-2/bax ratio a crucial factor in controlling caspase-dependent apoptosis [52]. Reduced Bcl-2 expression was observed [53].

The current study showed that there was a statistically significant difference between adopting zinc nanoparticles and a diode laser to prevent the progression of cancer by promoting the expression of the p53 gene. Additionally, the use of both nanoparticles and lasers affected the expression of the BAX gene, ramped up and increased the rate of the gene, and ultimately resulted in the death of cancer cells in the treatment of cancer cell lines employing BAX gene expression. However, after applying a combination treatment of laser and zinc nanoparticles, there was no visible alteration in the Bcl2 gene.

CONCLUSIONS

The outcomes of the study showed that there was a significant difference in combination therapy on the number of cells experiencing early apoptosis compared to

live, late apoptosis, and necrosis, it produced cell cycle arrest, apoptosis, and ROS levels raised in cancer cells. Malignant CaCo2 cells were inhibited by a combination therapy using zinc-oxide nanoparticles and diode laser, and there was a significant difference between the percentage of cell lines decreasing in the G2 phase of the cell cycle compared to the S and G1 phases. Highly noticed modifications and significant differences were reported when the proportion of cell lines in DCFH- compared to DCFH+ had been reduced using laser and zinc nanoparticles alone or together, the percent of cells in the control remained at high rates and decreased in all treated cases with different ratios. Considering all, the results suggest that the combination therapy may be a promising treatment approach for colorectal cancer, while it up-regulated the pro-apoptotic gene Bax and at the same time resulted in a downregulation of the anti-apoptotic gene Bcl-2. Also, this study demonstrates that the combination of zinc oxide nanoparticles and diode laser therapy showed a synergistic anticancer effect on colorectal cancer cells in vitro.

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AUTHOR CONTRIBUTIONS

The supervisor Assistant Prof. Dr. TAM made the main conception of writing a review, and the methodology, lab practical work, and data statistical analysis were done by the M.Sc. student CHSR. CHSR did article drafting while TAM provided final clearance for the paper to be published and critically edited it for important intellectual content. The final, published version of the work has been seen by all writers, who have also given their approval to it.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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