

The Photodynamic Therapy of N-TiO₂ and GOT Nanoparticles Enhances Chemotherapy Efficacy in Melanoma Preclinical Models

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Abstract

Introduction: Melanoma is the most aggressive form of skin cancer, with a rising incidence and poor prognosis in advanced stages. Due to its resistance to conventional therapies, alternative approaches such as photodynamic therapy (PDT) are under active investigation. This study evaluated the potential of N-doped TiO₂ (N-TiO₂) and graphene oxide–TiO₂ (GOT) nanoparticles (NPs), alone or in combination with cisplatin (CDDT), with or without xenon-light activation, to enhance chemosensitivity in melanoma preclinical models. **Materials and Methods:** F10 melanoma cells were treated with varying concentrations of N-TiO₂ and GOT NPs alone or combined with CDDT, under light and dark conditions. The viability and cytotoxicity of the cells were assessed. For in vivo experiments, tumors were induced in syngeneic mice, which subsequently received N-TiO₂, GOT, CDDT, or combination therapy with or without irradiation. After ten days of treatment, tumor volume reduction and pathological response were evaluated. **Results:** Photoactivated N-TiO₂ and GOT NPs significantly enhanced the cytotoxic effects of CDDT and reduced melanoma cell proliferation and tumor growth. In vivo, treatment responses ranged from 50–55% for N-TiO₂ + CDDT and 60–70% for GOT plus CDDT under PDT conditions. Histopathological analysis confirmed the absence of notable cytotoxic or metastatic effects on major organs, including the liver and spleen. **Conclusion:** Photoactivated N-TiO₂ and GOT nanoparticles potentiated the antitumor effects of cisplatin, reduced tumor proliferation, and enhanced therapeutic responses in melanoma preclinical models. These findings suggest that PDT with N-TiO₂ or GOT NPs, in combination with chemotherapy, may be a promising alternative strategy for melanoma management.

Keywords: Cisplatin, Melanoma, GOT nanoparticle, N-TiO₂ nanoparticle, Photodynamic therapy, Syngeneic mice

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Introduction

Melanoma is the most aggressive form of skin cancer, characterized by high metastatic potential and resistance to conventional treatments, and accounts for approximately 75% of skin cancer-related deaths [1, 2]. It is the fifth most common cancer in men and the sixth in women [3]. Despite advances in targeted therapies and immunotherapies, the management of advanced melanoma remains challenging. Surgical excision is the first-line treatment for early-stage disease; however, recurrence rates remain high [4]. Although chemotherapy is used

in advanced cases, it is often associated with substantial toxicity and reduced quality of life, underscoring the need for alternative therapeutic approaches that are effective, safe, and economically feasible [5]. Photodynamic therapy (PDT) is an emerging modality that has shown promising results in melanoma [6]. PDT relies on three key components-photosensitizers (PS), a light source, and molecular oxygen [7]. Upon absorption of light at a specific wavelength, the PS becomes activated and undergoes photochemical reactions that generate reactive

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oxygen species (ROS), including hydroxyl radicals, singlet oxygen, and superoxide anions. These highly reactive species interact with nucleic acids, proteins, and cellular membranes, leading to oxidative stress and cell death [8, 9]. Depending on cell type, PS, irradiation parameters, and oxygen availability, PDT can induce apoptosis, necrosis, or autophagy [10]. PDT can also be used as an adjuvant to chemotherapy, radiotherapy, or immunotherapy, enhancing tumor cell susceptibility without compromising the efficacy of these treatments [11].

Among various PSs, nanoparticles (NPs) have emerged as attractive candidates due to their non-invasive properties, ability to enhance oxidative stress, and effective induction of cell death. Metallic and metal oxide NPs, as well as metal-based nanoformulations doped with additional elements, have demonstrated PDT efficacy in both in vitro and in vivo cancer models [11, 12]. NPs are particularly appealing because of their strong absorption and scattering in the near-infrared region (NIR). In addition, some other characteristics, such as their ability to enter cells efficiently due to small size, reduced lymphatic clearance, and improved drug uptake, make them suitable for use in clinical settings [13, 14]. Titanium dioxide (TiO₂) nanoparticles have been widely studied as PSs owing to their low toxicity, biocompatibility, high stability, and photoreactivity [12]. However, their use is limited by activation predominantly under ultraviolet (UV) light, which is harmful for clinical applications. Doping TiO₂ with metallic or non-metallic elements can shift its absorption spectrum toward visible light, improving its suitability for PDT [15]. In particular, nitrogen-doped TiO₂ (N-TiO₂) and graphene-modified TiO₂ have shown enhanced photocatalytic activity under safer wavelengths [16]. Our previous work demonstrated that graphene oxide–titanium (GOT) nanoparticles possess strong PDT properties in human melanoma cells by increasing intracellular ROS generation and apoptosis [17]. Building on these findings, the present study aims to investigate the therapeutic potential of N-TiO₂ and GOT nanoparticles under PDT conditions to enhance the chemotherapeutic efficacy of cisplatin (CDDT) in a melanoma mouse preclinical model.

Materials and Methods

Preparation of NPs

N-TiO₂ and GOT nanoparticles (NPs) were prepared as mentioned in our previous studies [15, 17]. Briefly, N-TiO₂ NP was synthesized by mechanically blending urea with anatase form of TiO₂ powder with a weight ratio of 4:1. Then, it was heated for 1 hour to reach 400°C and then cooled to room temperature. The light-yellow powder obtained was finely ground in an agate mortar before being utilized [15]. Graphene oxide (GO) was initially prepared through the chemical exfoliation of industrial graphite and then was oxidized to graphitic oxide with the help of oxidants like H₂SO₄, HNO₃, H₂O₂, and KMnO₄. In the subsequent stage, a molar ratio of 70:30 was employed to fabricate the reduced GO/TiO₂ nanocomposite [17].

Dynamic light scattering (DLS) (Nanotracer Wave model, Microtrac Inc, X-ray diffraction (XRD), and electron microscopy (TEM) measurements were previously used to investigate and confirm nano-dimensions of N-TiO₂ NPs and GOT nanocomposites [15, 17].

For experimental use, the NPs were suspended in cell culture medium at a concentration of 1 (one) mg/mL and dispersed using a sonicator (JAC Ultrasonic 2010, Jeio Tech, South Korea) for 10 minutes. Immediately after sonication, fetal bovine serum (FBS) was added to each suspension at 10% (v/v) to prevent nanoparticle aggregation [15].

In vitro treatment

Mouse melanoma F10 cells were obtained from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% penicillin–streptomycin (Inoclon Co., Tehran, Iran), and maintained at 37 °C in a humidified incubator with 5% CO₂.

Cells were treated with various concentrations of N-TiO₂ (1, 10, 100, 200, and 400 µg/mL), GOT (1, 10, and 100 µg/mL), cisplatin (CDDT; 1, 10, 20, 50, and 100 µg/mL), or their combinations. Following treatment, cells were exposed to either irradiation or non-irradiation conditions and incubated for 24 hours. Photoactivation was performed using a PDT device equipped with a 55 W xenon lamp as the visible light source, delivering an irradiance of 20 mW/cm² for 10 minutes at a distance of 20 cm.

Cell Viability Assay

Cell viability was assessed using the trypan blue exclusion assay. F10 melanoma cells were treated with N-TiO₂ (1, 10, 100, 200, and 400 µg/mL), GOT nanoparticles (1, 10, and 100 µg/mL), or CDDT (1, 10, 20, 50, and 100 µg/mL), either alone or in combination. After 24 hours of incubation under PDT conditions (10 minutes of irradiation) or non-PDT conditions, both control and treated cells were stained with 0.4% trypan blue solution and counted using a hemocytometer. Unstained cells were recorded as viable, while blue-stained cells were counted as non-viable. Cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = (\text{Number of viable cells} / \text{Total number of cells}) \times 100$$

MTT Assay

The cytotoxic effects of the nanoparticles and CDDT were evaluated using the MTT assay. Briefly, 1×10⁴ cells/well were seeded and treated with various concentrations of N-TiO₂ (1, 10, 100, 200, and 400 µg/mL), GOT nanoparticles (1, 10, and 100 µg/mL), CDDT (1, 10, 20, 50, and 100 µg/mL), or their combinations under PDT (10 minutes of irradiation) and non-PDT conditions. After 24 hours of incubation, 10 µL of MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was added to each well, and plates were incubated for an additional 4 hours. Subsequently, 100 µL

of DMSO was added to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm using an ELISA microplate reader.

Cell viability and cytotoxicity were calculated using the following formulas:

Cell viability (%) = (Mean OD of treated cells / Mean OD of control cells) × 100

Cytotoxicity (%) = 100 – Cell viability (%)

In vivo experiments

The C57BL/6 male mouse strain was obtained from the Pasteur Institute of Iran and transferred to the animal care facility of Imam Khomeini Hospital. All the mice were 8-12 weeks old. They were kept under standard conditions in a 12-hour-light and 12-hour-dark cycle with free access to food and water. If they showed any signs of problems such as trouble in eating, drinking, reducing their activities, or unusual weight loss, they were immediately excluded from the study.

To generate the syngeneic mouse melanoma model, 10⁶ F10 mouse melanoma cells in 100 µl of DMEM media were prepared and injected into the flank of the mice. Following 2-3 weeks, the tumor was formed, and its volume was measured with the $\frac{1}{2} \times \text{length} \times (\text{width})^2$ formula every other day. When the tumor volume reached 100–300 mm³, the mice were randomly categorized into 8 groups, each containing five mice, and then chemotherapy was initiated. In this protocol, mice were intratumorally injected with 100 µL of normal saline as a control group, 100 µl of CDDT (2.3 mg/kg), N-TiO₂ NP (20 mg/kg, GOT NP (2mg/kg), and their combination under PDT and non-PDT conditions. The irradiation of the PDT group was performed under Xenon light for 10 min from a distance of 20 cm in a special device designed for this purpose. PDT was performed 10 min after injection. Body weights and tumor volumes were measured every other day until the end of the study. The injection was carried out every other day for 10 days, and then the tumors and vital organs, such as the liver and spleen, were removed by surgery and were kept in formalin for pathological analyses.

Pathologic complete response (pCR)

To assess the response to chemotherapy, the number of residual tumor cells present in tissue sections after treatment was quantified. A lower number of remaining tumor cells was considered indicative of a better therapeutic response. Pathological complete response (pCR) scoring was reported according to the following criteria: RX means that the presence of the residual tumor cannot be assessed; R0 shows no residual tumors were observed; R1 indicates some microscopic residual tumor was seen and reported as R1a to show less than 30%, R1b, 31–69%, and R1C more than 70% residual tumor was counted.

Hematoxylin and eosin (H&E) staining

Formalin-Fixed, Paraffin-Embedded (FFPE) tissues, including tumors, liver, and spleen, were prepared

according to standard protocol [18]. Then, 5–10 µm sections were cut by microtome, and stained with H&E routine lab protocol [19]. The hematoxylin stains the nucleus blue, and eosin makes the cytoplasm and connective tissues pink. So, mitotic cells can be easily counted in one mm² of a microscopic field of view. The mitotic index is widely used in pathology to evaluate the aggressiveness of a tumor and to improve prognosis and future patient management [20].

Statistical analysis

The results of each experiment were obtained from three independent experiments with three repetitions, and the mean ± standard deviation (SD) was measured. The statistical analyses were conducted by Student's t-test or ANOVA using SPSS software ver. 16 and/or GraphPad Prism 8.3.0. The p-value <0.05 was considered as statistically significant.

Results

N-TiO₂ and GOT NPs reduce proliferation in F10 melanoma cells under PDT more than in non-PDT conditions

The evaluation of the viability of the treated cells with different concentrations of N-TiO₂, GOT NPs, and/or CDDT using the trypan blue exclusion assay was performed under PDT and non-PDT conditions. As depicted in Figure 1, the PDT effect of NPs could reduce cell viability in a dose-dependent manner. Although monotherapy of CDDT decreased the rate of cell viability to 42.2% and 37.03% with high doses of 50 and 100 µg/ml, respectively (Figure 1a), PDT with low doses of CDDT (5, 10 µg/ml) showed impressive results. The combination of CDDT (10 µg/ml) with 400 µg/ml of N-TiO₂ NP reduced the cell viability to 30.03% in the presence of light (Figure 1b), while in the same situation, the effect of PDT N-TiO₂ monotherapy on viability was calculated at

Table 1. Pathologic Complete Response (pCR) and Mitotic Rate in N-TiO₂, GOT NPs, and/or CDDT under PDT and the non-PDT Situation in the Mouse Melanoma Model

Treatment group		pCR effectiveness (%)	Mitotic Rate (Mean)
N-TiO ₂	Light -	30-35	19
	Light +	35-40	16
GOT	Light -	30-35	20
	Light +	35-40	14
CDDT	Light -	40-45	16
	Light +	40-45	15
N-TiO ₂ /CDDT	Light -	45-50	17
	Light +	50-55	13
GOT/CDDT	Light -	55-60	12
	Light +	60-70	9
Control	Light -	0	29
	Light +	0	30

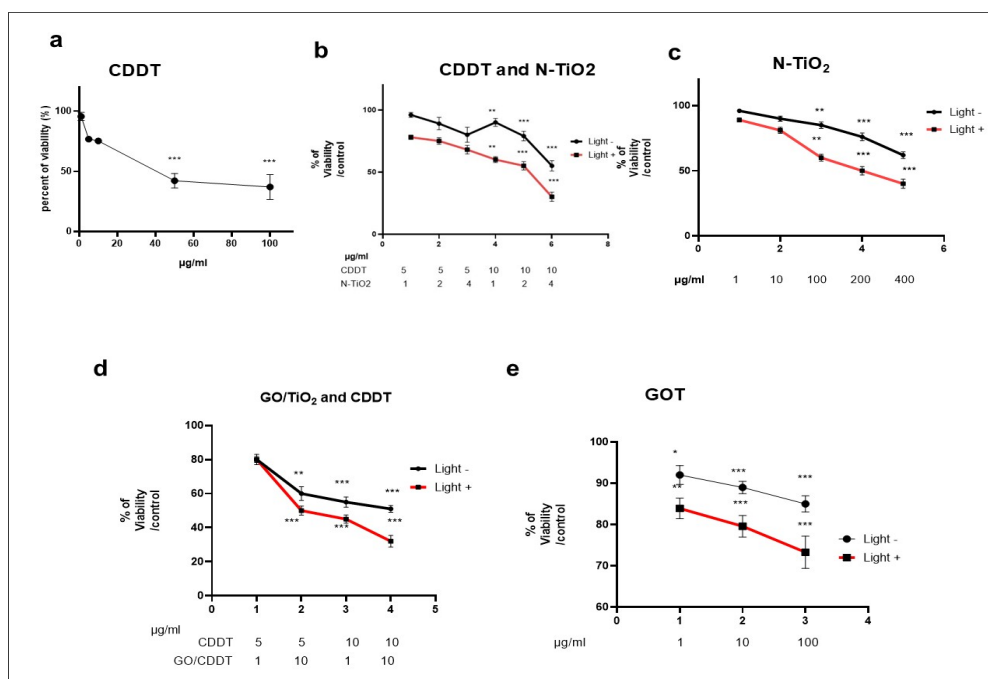


Figure 1. Effect of Different Concentrations of Cisplatin (CDDT) and/or N-TiO₂ and GOT NPs alone and in Combination on the Viability of F10 Cells. The cells were treated with various concentrations of A) CDDT (1, 10, 20, 50, and 100 µg/ml), B) CDDT (5, 10 µg/ml) and N-TiO₂ NPs (1, 200, 400 µg/ml), C) N-TiO₂ (1, 10, 100, 200, 400 µg/ml), D) CDDT (5, 10 µg/ml) and GOT (1, 10 µg/ml), E) GOT NP (1, 10, 100 µg/ml) for 24 h under PDT and non-PDT situations, then viability was studied using trypan blue exclusion test. The results are from triplicate measurements across three independent experiments and are represented as means ± SD. Statistically different results are indicated with * (p<0.05), ** (p<0.01) and *** (p<0.001) compared to untreated cells.

40.05% (Figure 1c). The combination of CDDT with GOT NP also displayed almost the same results. As depicted in Figure 1d, 10 µg/ml of CDDT with 1 and 10 µg/ml of GOT NPs decreased the cell viability to 45% and 32%, respectively. The results also demonstrated that the cell viability of GOT with 1 and 10 µg/ml doses alone in the presence of light was 79.6% and 73.3%. The high doses of GOT (100 µg/ml) did not represent the highest cytotoxicity (Figure 1e).

MTT assay was performed to determine the cytotoxicity of the N-TiO₂, GOT NPs, and CDDT chemotherapy. As shown in Figure 2, the cytotoxicity of CDDT and NPs was dose-dependent, and the PDT enhanced the cytotoxicity effects of CDDT when used with N-TiO₂ and GOT NPs. For instance, the cytotoxicity of N-TiO₂ with 100 and 400 µg/ml concentrations was 48.23% and 56.04% in combination with CDDT and PDT situations, respectively (Figure 2a), while in monotherapy it was 25.13% and 38.07%, respectively (Figure 2b). The cytotoxicity of GOT NPs also showed better results in combination with CDDT. As 10 µg/ml of GOT NP and 10 µg/ml CDDT enhanced the cytotoxicity to 56% in front of PDT (Figure 2c), while GOT NP just showed the 19% cytotoxicity in PDT (Figure 2d).

N-TiO₂ and GOT NPs in combination with CDDT reduce tumor growth and enhance drug response in syngeneic melanoma mice under PDT conditions

To confirm the effect of N-TiO₂, GOT NPs, and CDDT drugs under PDT conditions, the syngeneic mouse model was generated and treated with the mentioned drugs.

The proper doses of drugs were obtained by repeated experimental trials with significant responses to treatment and no toxicity on vital organs such as the spleen and liver.

Although the nano-drugs and CDDT could reduce the tumor volume and tumor size in mice, the PDT situation showed a significant reduction in the tumor size and tumor volume (Figure 3). As depicted in the figures, the role of PDT in the reduction of tumors was obvious when treatment with GOT and N-TiO₂ NPs was compared to non-PDT usage; however, the role of GOT is more prominent than N-TiO₂ (Figure 3a). The combination therapy of CDDT and NPs could decrease the tumor growth significantly (p<0.001). As PDT of CDDT/GOT and CDDT/N-TiO₂ could reduce the tumor growth to 1523.02 mm³ and 2560/369 on the last day of treatment in comparison to 3221.56 mm³ and 5656/126 mm³, respectively in the absence of light (p<0.001) (Figure 3b). The morphology of tumor was shown in Figure 3c to display the size of tumor and comparison between PDT and non-PDT situation.

H&E staining demonstrated a decrease in mitotic rate in N-TiO₂, GOT NPs, and CDDT treatment and/or in combination under PDT in comparison with non-PDT. While the mitotic rates in N-TiO₂ and GOT NPs were 16 and 14, respectively, the combination of them with CDDT was 13 and 9, respectively, which presents the power of PDT in the treatment of melanoma (Table 1). To find out whether there is a complete response after chemotherapy, the presence of residual tumor cells was checked in tissue samples of melanoma mice. As can be observed in Figure 4 and Table 1, the best effectiveness

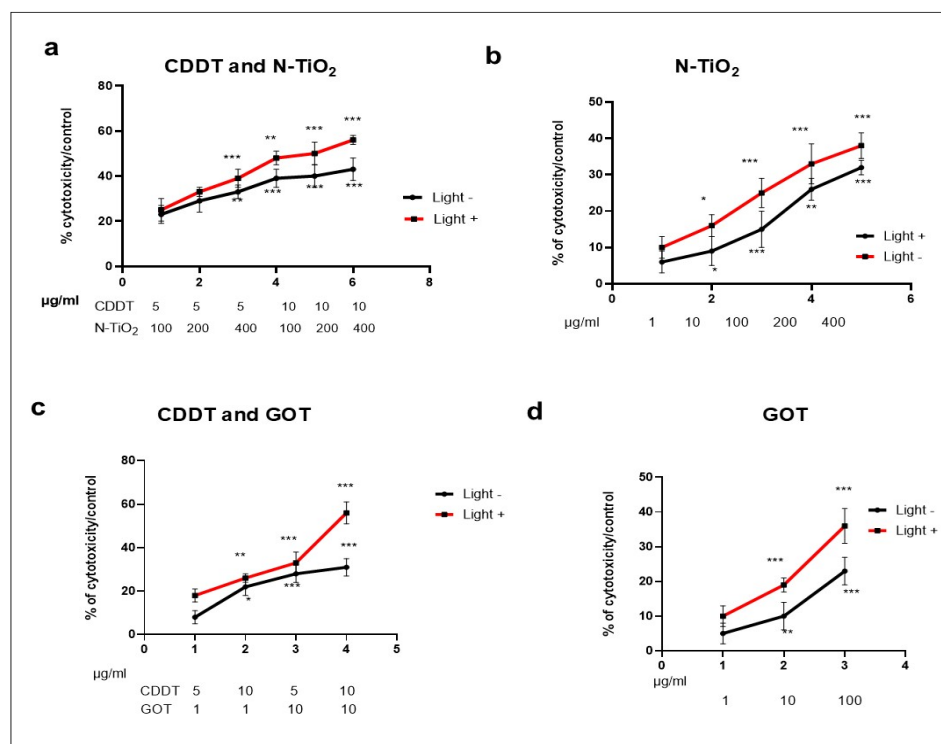


Figure 2. Effect of Different Concentrations of Cisplatin (CDDT) and/or N-TiO₂ and GOT NPs alone and in Combination on the Cytotoxicity of F10 Cells. The cells were treated with various concentrations of A) CDDT (5, 10 µg/ml) and N-TiO₂ NP (100, 200 400 µg/ml) B) N-TiO₂ NP (1, 10, 100, 200, 400 µg/ml) C) CDDT (5, 10 µg/ml) and GOT (1, 10 µg/ml), D) GOT NP (1, 10, 100 µg/ml) for 24 h under PDT and non-PDT situations, then cytotoxicity was studied using MTT assay. The results are from triplicate measurements across three independent experiments and are represented as means ± SD. Statistically different results are indicated with * (p < 0.05), ** (p < 0.01) and *** (p < 0.001) compared to untreated cells.

of the group was achieved by the prescription of GOT NP and CDDT, which resulted in 60-70% responses in the presence of PDT.

The H&E staining confirmed that no signs of metastasis or cytotoxicity were observed in the mice's tumors or organs such as the liver and spleen (Figure 5) following chemotherapy with N-TiO₂ and GOT NPs and/or cisplatin. In addition, the tumor volume reduction was positively related to the decreased proliferation and mitotic cell counting in treated mice under PDT.

Discussion

Photodynamic therapy (PDT) has emerged as a promising alternative approach for the treatment of melanoma, a malignancy known for its resistance to conventional therapies [21, 22]. Among the various photosensitizers (PSs) used in PDT, nanoparticles (NPs) have shown great potential; however, their intrinsic cytotoxicity or limited activation range often require structural modification to improve safety and therapeutic efficiency. For example, TiO₂ nanoparticles exhibit cytotoxic effects at high concentrations and require ultraviolet (UV) irradiation for activation, which restricts their medical applicability [23]. Doping TiO₂ with non-metallic or carbon-based molecules such as nitrogen or graphene improves its optical activity under visible-light wavelengths, making these modified NPs more suitable for PDT applications [24, 25].

In this study, N-TiO₂ and GOT nanoparticles were evaluated as PSs based on previous findings demonstrating their ROS-generating and pro-apoptotic effects on melanoma cells under visible-light PDT [15, 17]. Upon irradiation, these NPs induce various forms of cell death, including apoptosis and autophagy [11, 26]. The effectiveness of PDT was evaluated by measuring cell viability and cell toxicity post-treatment and irradiation of the cells compared to non-irradiation. The GO NP prevents the growth of cells and leads them to death only under visible light irradiation [16, 27]. Furthermore, the GOT NP has been shown to have more power than TiO₂ NP alone [28]. In line with these results, GOT NPs and xenon light were used in this experiment to reduce the proliferation of melanoma cells. The results have shown that N-TiO₂ and GOT NPs as PS could decrease the proliferation and increase the cytotoxicity following irradiation in melanoma mouse cells, respectively (Figure 3).

Although PDT is effective, its therapeutic window can be limited by potential collateral effects within the tumor microenvironment. Therefore, combination approaches particularly PDT combined with chemotherapy have been recommended to enhance tumor control while reducing chemotherapy-associated toxicity [29]. The results of this study were confirmed by a significant reduction in the proliferation of PDT-treated cells as compared to the non-PDT-treated cells, as shown in Figures 1 and 2. This kind of treatment is promoted when a combination of NPs and chemotherapy is applied together. As a consequence, it

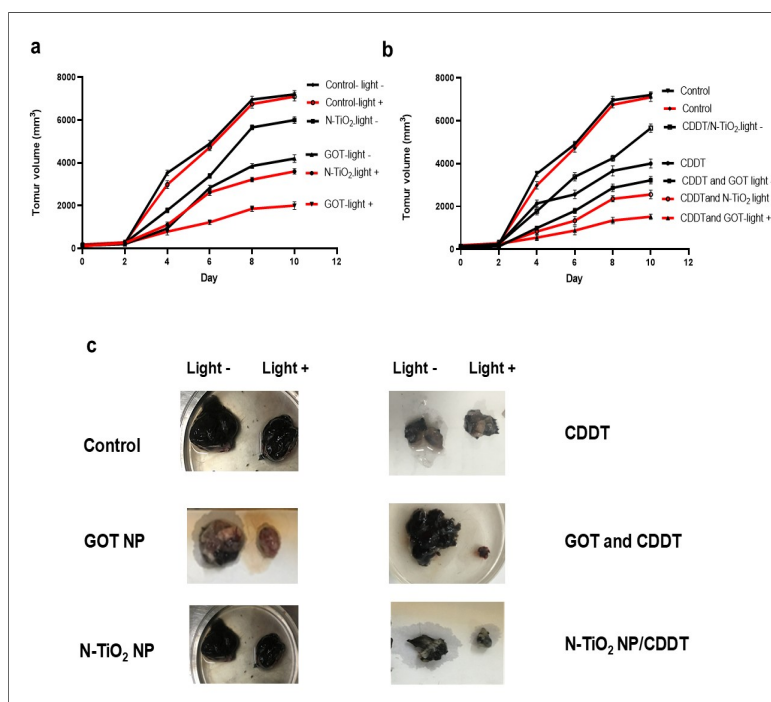


Figure 3. The Effect of N-TiO₂, GOT NP, CDDT, and their Combination on Tumor Volume in C57BL/6 Mice Under PDT and non-PDT Situations (a, b) and Tumor Size (c). a) Mice Treated with CDDT (2.3 mg/kg), N-TiO₂ NPs (20 mg/kg), GOT NPs (2 mg/kg), and b) a Combination of them. Developed tumors showed a significant reduction in tumor volume compared with the control and monotherapy. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ are considered as significant data. c) The tumors were surgically removed and compared in two PDT and non-PDT conditions.

appears that the partitioning of the NPs as a photosensitizer is more important than chemotherapy alone in the efficacy of the PDT treatment. NPs are good candidates as PSs and can be used in conjugation with drugs and/or accompanied by them. In both situations, they have shown a better outcome than monotherapy [8, 30].

The in vivo consequences are in excellent agreement with those obtained from in vitro tests: as shown in Figure 3, the PDT effects are markedly more extensive in nano drugs and chemotherapy injection, which causes a tumor response, than that observed in the absence of light. The application of CDDT along with NPs also leads to a significant decline in tumor volume relative to non-PDT conditions. A lower concentration of the N-TiO₂ (20 mg/kg) or GOT (2 mg/kg) NPs photosensitizer in combination with CDDT (2.3 mg/kg) showed no toxicity and suitable biodistribution of drugs. Thus, a significant reduction in tumor growth is obtained only with an appreciable dose of PSs in the presence of light. The other factors that affect tumor reduction are the penetrability of the light through the tissue, the suitable light distance, and the kind of wavelength of the light [31]. The penetration of light into the tissues activates the PS to produce ROS and damage the cancerous cells. The suitable distance should be regulated so as not to cause damage to normal tissues, and in this experiment was achieved at 20 centimeters. Furthermore, visible light has a suitable wavelength with no danger to normal tissues [32]. Using LED light or blue light as a normal wavelength was reported in the PDT of the murine melanoma model, which showed a tumor reduction compared to the control [33, 34]. It is also intended to minimize the accumulation and toxicity of

nanomedicines as PSs in the endothelial system, such as the liver and spleen [35-37]. H&E staining of these organs post-treatment showed no toxicity or aggregation of NPs (Figure 5), which could induce a more efficient tumor response to PDT. The response to treatment also supports the effect of PDT on NPs and the combination therapy.

To reduce drug resistance and toxicity of NPs [38], we applied PDT of CDDT with lower doses in combination with the mentioned NPs, which confirmed our in vitro results and showed a decrease in tumor volume in mouse melanoma models. The optimal protocol for tumor and targeted therapy can destroy melanoma cells and increase the immune response, thus raising the efficiency of treatment [39]. PDT combination with chemotherapy and functionalized metal nanostructures offers promising solutions to the side effects of chemotherapeutic drugs. The nanodrugs are capable of entering the cells easily with no induction of immune cells, encapsulating the medicine, or binding to them, responding to irradiation, and thus exhibiting lower drug toxicity with increasing efficiency [40]. As a consequence, PDT-based NPs are applied not only to increase the effect of chemotherapy but also to deliver it to the target tissues [41, 42]. In this experiment, PDT-based NPs are accompanied by CDDT chemotherapy to target melanoma tumors under visible light and show the booster effect of PDT and NPs in modern treatment.

In conclusion, drug resistance remains a major challenge in melanoma treatment. Agents capable of overcoming this barrier and promoting tumor cell death are of high clinical interest. Our findings demonstrate that combining low-dose cisplatin with N-TiO₂ and GOT nanoparticles under PDT conditions significantly enhances

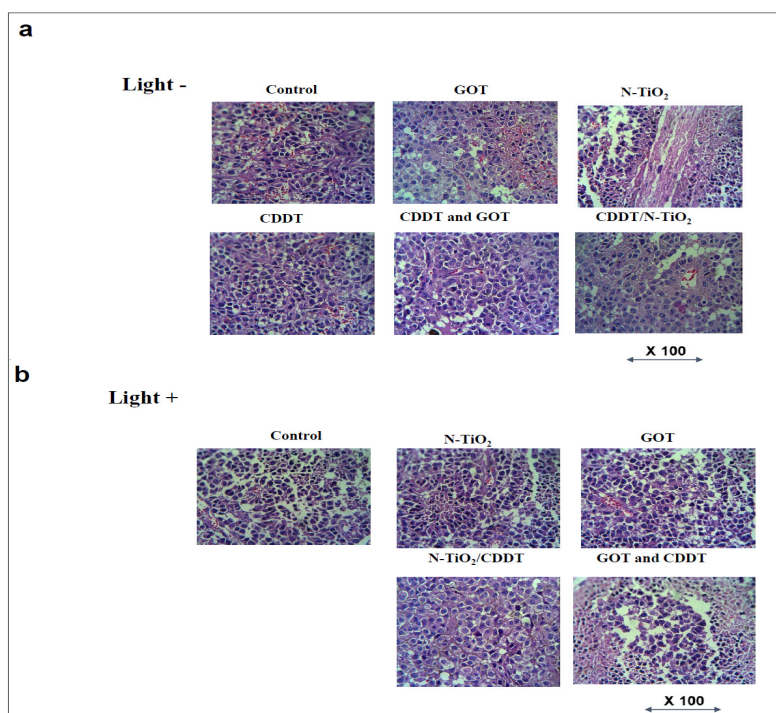


Figure 4. The Pathologic Complete Response (pCR) after Chemotherapy by CDDT and/or NTiO₂, GOT NPs alone, and a Combination of them on C57BL/6 mice. a) Under non-PDT; b) Under PDT Situation, the pCR of Drugs under Microscopy after H&E Staining

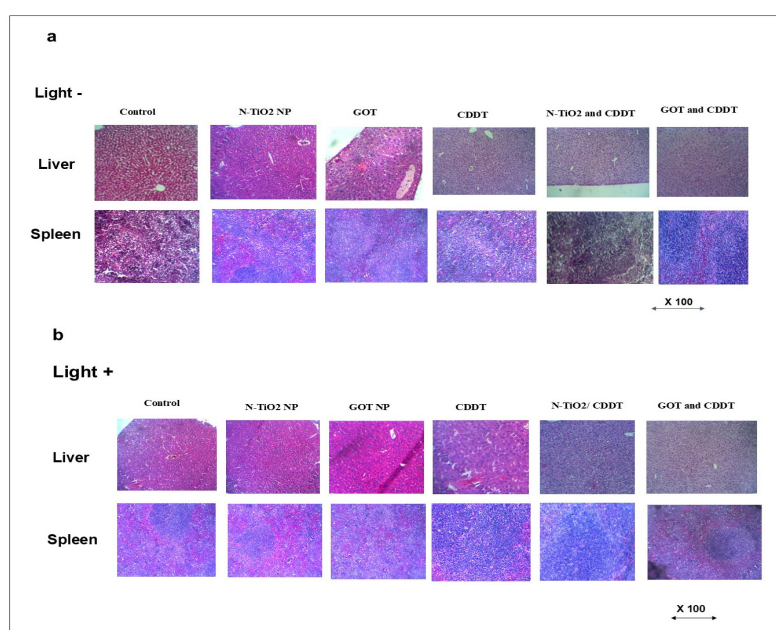


Figure 5. The H&E Staining of the Liver and Spleen of Tumors. a) under non-PDT and b) under PDT situation

antitumor efficacy in vitro and in vivo. Therefore, PDT using these nano-photosensitizers, in conjunction with chemotherapy, represents a promising therapeutic strategy for melanoma and warrants further investigation.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The project was approved by the Research Ethics Committee of Laboratory Animals - Tehran University of Medical Sciences with IR.TUMS.AEC.1401.003 approval ID.

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