UV Irradiation Enhanced In-Vitro Cytotoxic Effects of ZnO Nanoparticle on Human Breast Cancer

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Abstract. Cancer is a disease characterized by abnormal cell growth in body tissues. Generally, cancer can be treated with surgery, chemotherapy, radiation therapy, and many others. However, these treatments have side effects on healthy cells. ZnO nanoparticle is one of considerable interests in targeting pharmaceutical for the treatment of cancer by inhibiting the cell proliferation. Therefore, in this study, we investigated In vitro cytotoxic effects of ZnO nanoparticles on the growth of MCF-7 breast cancer cells. The cytotoxic test was performed using varying concentration of ZnO nanoparticles (0, 25, 50, 75, 100 μg/mL) with different times of UV irradiation (0, 90, 180, and 270 seconds). The cytotoxic effect was evaluated by measuring the absorption of formazan crystal at wavelength of 490 nm using ELISA reader and converted to determine the percentage of living cells. The surviving cancer cells are characterized by their ability to convert MTT salts in to formazan crystals. The concentration of ZnO nanoparticles cause the decrease in viability of MCF-7 cells, with the lowest viability at ZnO 100 μg/ml concentration of 57.5%. UV radiation increased the toxicity effect of ZnO nanoparticles on MCF-7 cells. A combination of ZnO 100 μg/ml nanoparticles with UV radiation exposure for 180 seconds had the lowest viability of the MCF-7 cells of 13.58%.

Keywords: Cancer, ZnO, UV irradiation, cytotoxic

1. Introduction
Cancer on the reproductive system, such as the mammary glands, is the leading cause of death among women worldwide. The female reproductive organ that possibly gets cancer is breast [1]. Breast cancer is the one of cancers widely studied and it becomes the most common cause of cancer-related deaths in women worldwide [2]. Based on GLOBOCAN (IARC) data in 2012, it is known that breast cancer is a cancer with the highest percentage of new cases in the female population in the world, that was 43.3%, and the percentage of deaths due to breast cancer was 12.9%. In Indonesia, the estimated incidence of breast cancer is 40 per 100,000 women [3].

The most common cancer treatments include surgery, radiation, and chemotherapy [4]. However, these treatments have adverse effects with high levels of morbidity in the form of normal tissue damage that can kill and damage healthy cells [5], and have toxic effects on healthy cells [4]. Photodynamic therapy (PDT) is a new method in the treatment of cancer. PDT has been proposed as a
minimally invasive therapeutic procedure, utilizing three important elements to induce cell death, namely photosensitizer (PS), light with a certain wavelength, and oxygen molecules [6]. PDT has no long-term side effects when compared to surgical procedures, chemotherapy or radiotherapy [7]. ZnO nanoparticle as a photosensitizer has an excellent chance to be utilized in the photodynamic (PDT) therapy with its ability as an anticancer agent. The previous study showed that, for example, Li et al. (2010) used UV radiation to increase the strength of ZnO nanoparticles to suppress the growth of hepatocellular carcinoma SMMC-7221 cancer cells [8]. A ZnO nanoparticle is a semiconductor material with a wide band-gap, that can readily absorb UV rays [9].

ZnO nanoparticles have been used in various applications in cancer therapy, biosensing, drug/gene delivery, nanomachines, and biomedical applications [9]. Akhtar et al. (2012) reported that ZnO nanoparticles have toxic effects on cancer cells (human hepatocellular carcinoma HepG2, human lung adenocarcinoma A549, and human epithelial bronchiolus BEAS-2B), but not toxic to healthy cells (astrocytes and hepatocytes) [10]. This study aimed to determine the effect of ZnO nanoparticles with UV radiation exposure on breast cancer cells (MCF-7) evaluated with the test of cytotoxicity by using 3-(4,5-dimethylthiazol-2-yl) reagents is 2,5-diphenyl tetrazolium bromide (MTT).

2. Materials and Methods

2.1. Cell Culture

Michigan Cancer Foundation-7 (MCF-7) cells (purchased from Sentral Ilmu Hayati Laboratory Universitas Brawijaya) were maintained in Roswell Park Memorial Institute-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Sigma, USA), 10% penicillin (Sigma, USA)-streptomycin (Sigma, USA) and were incubated in 5% CO$_2$ at 37°C. When the cells were confluent, the experiment could be started.

2.2. ZnO Nanoparticles

ZnO nanoparticles were purchased from Mineral and Advanced Material Laboratory FMIPA Universitas Negeri Malang, and dissolved in culture medium with the concentration of 0, 25, 75, and 100 μg/mL.

2.3. MTT-Assay

The effect of different ZnO nanoparticles concentrations on MCF-7 cancer cells was observed by MTT assay. The ZnO nanoparticles were at the concentrations of 0, 25, 75, and 100 μg/mL with different times of UV irradiation at 0, 90, 180, and 270 seconds. Initially, 1x10$^4$ cells were seeded into each well containing 200 μL cell culture medium in 96-well plate and incubated for 24 hours. On the next day, the cell culture was exposed to ZnO nanoparticle with serial concentration and followed by UV irradiation at different times exposure. The cells were incubated in 5% CO$_2$ at 37°C for 24 hours and 48 hours. At the end of the incubation, the cells were washed using PBS. Then, 200 μL MTT solution (5 mg/mL) was added into the well and were incubated for 4 hours until forming formazan crystals. The formation of formazan crystals was observed under an inverted microscope (Olympus IX-71, Japan), and absorption of formazan crystal was measured at wavelength of 490 nm using ELISA reader (Biorad), then the absorbance value was converted to determine the percentage of living cells. The placebo was cultivated under the same conditions without addition of ZnO nanoparticles and UV irradiation.

3. Results and Discussion

The cytotoxic effects of ZnO nanoparticles were determined by the MTT-assay method. The formation of formazan crystal in the cell culture is shown in Figure 1. The cell viability number was equal to the amount of formazan crystal within the cell and associated with the higher metabolic rate of the cell. [11].
Figure 1. Formation of formazan crystal on MCF-7 cells after treatment with ZnO nanoparticles and UV radiation for 90 s at 48 hours incubation; (A) ZnO nanoparticles with the concentration of 0 μg/ml, (B) ZnO nanoparticles with the concentration of 25 μg/ml, (C) ZnO nanoparticles with the concentration of 50 μg/ml, (D) ZnO nanoparticles with the concentration of 75 μg/ml, (E) ZnO nanoparticles with the concentration of 100 μg/ml; 400x.

The data analysis in Figure 2 shows an increase in the concentration of ZnO nanoparticles (without UV irradiation) causing the decrease in viability of MCF-7 cells, with the lowest viability at ZnO 100 μg / ml concentration of 57.5%. This result corresponds the study of Taccola et al. (2011), who reported that ZnO nanoparticles had selective cytotoxic properties against cell proliferation in benign and malignant tumors [12]. Mathuraman et al. (2014) also reported that ZnO nanoparticles could alter 3T3-L1 cell morphology, and increase cell death at the highest concentration of ZnO [13]. Elevated levels of intracellular ZnO can enhance cytotoxicity through zinc-mediated protein activity disequilibrium and oxidative stress. ZnO nanoparticles can induce oxidative stress in cancer cells, due to the semiconductor nature of ZnO induces ROS generation [9]. Production of reactive oxygen species (ROS) results in oxidative stress and cellular toxicity, which leads to DNA damage and cell death when the anti-oxidative capacity of the cell is exceeded [14].
UV radiation increased the toxicity effect of ZnO nanoparticles on MCF-7 cells, as indicated by the decreased viability of MCF-7 cells with exposure to UV radiation for 90, 180, and 270 s (Figures 2 and Figure 3). On 24-hour incubation, a combination of ZnO 100 μg / ml nanoparticles with UV radiation exposure for 180 seconds had the lowest viability of the MCF-7 cells of 13.58%. While at 48 hours incubation, the lowest cell viability of 13.61% was demonstrated at the same dose of ZnO nanoparticles, but with exposure to UV radiation for 90 seconds.

Figure 2. The level of cell MCF-7 viability after treatment with ZnO nanoparticles and UV irradiation at 24 hours incubation; the values are presented as mean ± SD.

Figure 3. The level of cell MCF-7 viability after treatment with ZnO nanoparticles and UV irradiation at 48 hours incubation; the values are presented as mean ± SD.
UV light is a form of electromagnetic radiation that can induce apoptosis through various molecular pathways [15]. UV radiation exposure can produce oxidative free radicals that are capable of interacting with macromolecules such as proteins, lipids, RNAs, and DNAs that alter the structure and disrupt the function of the macromolecule. UV light is efficient in generating ROS that can damage DNA through indirect photosensitization reactions, and can also be absorbed directly by the DNA that causes the mutation [16].

ZnO has a light-capturing group called chromophores, which will generate excess energy when absorbing UV light at specific wavelengths, resulting in the unstable ZnO structure. Hence, there will be electron transfer (e') from the valence band to the ZnO conduction band, simultaneously producing a hole (h') in the valence band. There is a charge imbalance so that ZnO is immediately neutralized by the H2O molecule to form a hydroxyl radical (OH·) which is free radical. According to Konopka and Goslinski (2007), the formed free radicals are very active and can damage proteins, lipids, and other cellular components, which can destroy cancer cells [17].

ZnO nanostructures are well potential photosensitizers based on their phototoxic action upon UV irradiation. ZnO nanoparticles are reported to exhibit a robust preferential ability to kill cancerous cells compared to healthy cells, and able to induce excessive production of reactive oxygen species (ROS) in various cell lines, resulting in oxidative stress and cellular toxicity [14]. The ZnO nanoparticles were effective to decrease the viability of cancer cells, and UV irradiation could significantly enhance the cytotoxic effect on cancer.

4. Conclusion
ZnO nanoparticles were useful to decrease the viability of MCF-7 cells, in the amount of 57.5% at ZnO 100 μg/ml concentration. UV radiation could enhance the ability of ZnO nanoparticles against MCF-7 cells of equal level, with reduced viability to 13.58%. The combination of ZnO nanoparticles and UV radiation can be developed as a method of cancer therapy on an in-vivo scale. ZnO nanoparticles potentially becomes an anti-cancer agent in the reproductive system, especially breast cancer cells.

Acknowledgments
We would like to thank Helly Nurul Karima, S.Pt., M.P. at LSIH-UB who has provided support for the development of this research as well as to other technicians.

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