学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Antibacterial activity and cytotoxicity *in vitro* of green-synthesized silver nanoparticles using *Brassica rapa* var. *japonica* leaf
（ミズナの葉を用いてグリーン合成された銀ナノ粒子の抗菌活性および試験管内細胞毒性）

Recently, NPs are widely used in several sectors such as in human health appliances, industrial fields, medical applications, biomedical fields, engineering, electronics, and environmental studies. Among all of the nanoparticles, Ag-NPs is being one of the most demandable material due to its enormous applications in versatile sectors. Now-a-days it has become a lucrative material in consumer products. At the same time conventional method of Ag-NPs synthesis is a concern of increasing environmental pollution. Already enormous study on the cytotoxic effect of Ag-NPs has been carried out in vitro and in vivo. Thus, green synthesis of Ag-NPs could be an alternative to overcome the emerging situation and researchers are now concern to have alternative synthesis processes such as biosynthesis, phyto-synthesis, and so on. Therefore, this research has been carried out with some specific objectives majorly green synthesis of Ag-NPs and exploration of their biomedical applications and the underlying mechanism.

In recent era versatile applications of silver nanoparticles (Ag-NPs) have been elevated. According to the requirements by different parties, tremendous amounts of Ag-NPs have been synthesized using conventional method. However, conventional method for synthesis of Ag-NP has been pointed out a matter of concern due to toxicity and human health implications as mentioned earlier. Therefore, it was hypothesized that the green synthesis of Ag-NPs could be regarded as safer synthesis method for green Ag-NPs. On the other hand, Ag-NPs are reported to have potential antitumor and anticancer properties in both in vitro and in vivo experiments. From above viewpoints, this study aimed to be green synthesis of Ag-NPs and evaluation their biomedical applications with underlying mechanisms. To achieve the purpose actual objectives were set. First, Ag-NPs were successfully synthesized from the reduction of Ag+ using AgNO3 solution as a precursor and Brassica rapa var. nipposinica/japonica leaf extracts as a reducing and capping agents. In the synthesis procedure no additional chemical reductant and stabilizing agents were used. The characterization of Ag-NPs was carried out using UV-vis spectrometry, energy dispersive X-ray (EDX) spectrometry, fourier transform infrared (FT-IR)

spectrometry, field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), atomic absorption spectrometry (AAS), and transmission electron microscopy (TEM). The analyses data revealed the successful synthesis of nano-crystalline Ag possessing more stability than commercial Ag-NPs. To confirm synthesis of Ag-NPs exhibiting less toxicity with high antibacterial activity, following experiments have been done. The cytotoxicity of Brassica Ag-NPs was compared with commercial Ag-NPs using PC12 cell system. Three ppm of commercial Ag-NPs reduced cell viability to 23% (control 97%) and increased lactate dehydrogenase activity, whereas, Brassica Ag-NPs did not show any cytotoxicity on both parameters up to a concentration level of 10 ppm in PC12 cells. Moreover, Brassica Ag-NPs exhibited inhibition zone of against growth of Escherichia coli (11.1 ± 0.5 mm) and Enterobacter sp. (15 ± 0.5 mm) which was higher than other green-synthesized Ag-NPs reported previously. The less cytotoxicity and high antibacterial activity of green synthesized Ag-NPs will be great benefits for the safe use of Ag-NPs in consumer products. On the basis of results in this study it could be concluded that cytotoxicity of Ag-NPs is depended on the stability of the particles and the stability depends on the encapsulation or coating of the surface of the particles. Therefore, it was considered that reaction temperature during synthesis could play a vital role in coating of the particles. From the results, it was tried to synthesize optimal Ag-NPs using Brassica rapa var. nipposinika/japonica leaf extract with various temperatures. The synthesis of Ag-NPs was done at four different temperatures such as 25 °C (room temperature), 60 °C, 80 °C and 100 °C in order to evaluate the extent of encapsulation of Ag-NPs. The synthesized Ag-NPs were again characterized using UV-vis. spectrophotometer, EDX spectrometer, XRD spectrometer, TEM, and dynamic light scattering techniques. The adopted characterization techniques clearly demonstrate that at 100 °C almost all particles were found to be encapsulated which was the primary objective of the present study.

Furthermore, in this study, the behavior of various concentrations of green synthesized Ag-NPs in cancer cells was clarified. Brassica Ag-NPs exposed to Caco-2 cells showed significant decrease of the cell viability, increase of the LDH activity in the medium, and decrease of intracellular GSH amounts. From these results, it is indicated possibility of anticancer ability due to Brassica Ag-NPs. Further study will be designed to unveil the mechanism behind the anticancer properties of Brassica Ag-NPs in Caco-2 cells.

In conclusion, this study clearly reveals the potentiality of Brassica leaf extract for the environment friendly green synthesis of Ag-NPs which can be encapsulated with optimal temperature. In addition, Brassica Ag-NPs are less toxic in comparison of commercial Ag-NPs with high antibacterial activity, and also have a possibility of anticancer ability.