www.vbripress.com/aml, DOI: 10.5185/amlett.2016.6134 Published online by the VBRI Press in 2016

Tumor-targeting hederagenin-loaded magnetic nanoparticles for anti-cancer drug delivery

Kwon-Jai Lee[1], Jeung Hee An[2*], Jae-Soo Shin[1], Dong-Hee Kim[3], Kang-Hyun Chung[4]

[1]*Department of Advanced Materials Engineering, Daejeon University, Daejeon 300-716, Korea*
[2]*Division of Food Bioscience, Konkuk University, Chungju, 27478, Korea*
[3]*Department of Pathology Lab, College of Oriental Medicine, Daejeon University, Daejeon, 300-716, Korea*
[4]*Department of Food Science and Technology, Seoul National University of Science & Technology, Seoul 139-743, Korea*

[*]Corresponding author. Tel: (+82) 43-840-3584; E-mail: anjhee@hanmail.net

Received: 02 September 2015, Revised: 05 February 2016 and Accepted: 20 March 2016

ABSTRACT

In this study, the anti-tumor activity of hederagenin-loaded magnetic nanoparticles (HMP) was examined in cancer cells. Composite nanoparticles with an average size of 32.5 nm were prepared using a chemical co-precipitation technique. The characteristics of the particles were determined via X-ray diffraction, field emission scanning electron microscopy, attenuated total reflectance fourier transform-infrared spectroscopy, and energy-dispersive X-ray spectroscopy. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay showed that the magnetic nanoparticles were non-toxic against cancer. In particular, HMPs were cytotoxic at 73.12 % breast cancer (MCF-7), at 70.2 % against prostate cancer (DU145 cells), at 72.15 % against neuroblastoma cancer cells (U87), at 579.15 % in human brain cancer cells (SH-SY5Y), and at 74.5 % in human cervical cancer cells (HeLa) at 250 μg/mL. Our results demonstrated the biological applicability of HMPs as anticancer agents and as agents for enhanced drug delivery against human prostate cancer cells. Our results indicate that the magnetic nanoparticles were biostable and that HMPs functioned effectively as drug delivery vehicles.

Keywords: Magnetic nanoparticles; hederagenin; drug delivery; anti-tumor activity.

Introduction

Nanotechnology has emerged as a very important field in medicine and more specifically in targeted drug delivery [**1**]. The therapeutic efficiency of drugs currently being used may be improved if they were more efficiently delivered to their biological targets through the appropriate application of nanotechnologies [**2**]. The fundamental objective of nanomaterial-based cancer therapy relies on overcoming the major limitations of conventional cancer therapy (chemotherapy or radiotherapy), such as toxicity to normal cells [**3**]. The best way to increase the efficacy and reduce the toxicity of any drug is to direct the drug to its target and maintain its concentration at that site for a sufficient amount of time for therapeutic action [**4**]. Given the useful properties of nanosized materials, several applications have been developed using these materials, such as drug delivery, imaging, drug targeting, and anticancer activity [**5**]. Based on the physicochemical properties of nanomaterials, several methods such as magnetic hyperthermia, nanocryosurgery, sonodynamic therapy, and sonophotodynamic therapy have developed in the recent years for efficient cancer therapy [**6**]. Moreover, nanomaterials can be easily functionalized with several polymers to encapsulate drugs/photosensitizers for drug delivery applications in chemotherapy or photodynamic therapy [**7**].

Particularly, magnetic nanoparticles are used widely in a number of biomedical applications and are one of the most commonly used magnetic materials because of their strong magnetic properties and low toxicity [**8**]. Magnetic-targeted drug carriers, which are a major research focus in drug targeting, are prepared to be magnetic or as a magnetic core coated with biocompatible drugs for delivery [**9**].

Drug-loaded magnetic nanoparticles have been evaluated for a variety of biomedical applications, including DNA separation, drug delivery, magnetic resonance imaging, hyperthermia, and cell labeling [**10**]. To employ these magnetic nanoparticles in biomedical applications, the particles must frequently first be modified with biocompatible compounds [**11**]. Researchers have achieved this by either coating the magnetic nanoparticles with a layer of biodegradable polymers or by distributing a polymer matrix evenly throughout the nanoparticles [**11, 12**].

Using these approaches, magnetic drug targeting has been successfully employed to improve localized drug delivery and to enhance drug-therapeutic efficiency in various tumor types [**12, 13**]

Hederagenin, a group of triterpenoid saponins [**14**] are widely used as medicines in many Asian countries for their anti-inflammatory, antibacterial, anti-platelet aggregation, antibacterial, and neuroprotective activities [**15-17**]. Recently, a number of studies have demonstrated the cytotoxic and anti-tumor activities in tumor cells [**18-19**]. However, hederagenin has not been tested in many other cancer types. Therefore, the use of hederagenin-coated magnetic nanoparticles should be evaluated to determine the efficacy of the drug against cells.

In this study, we evaluated the optical characteristics of magnetic nanoparticles loaded with ethylene glycol and a hederagenin and evaluated their anticancer activities against human brain cancer cells (SHSY5Y), human cervical cancer cells (HeLa), neuroblast cancer cells (U87), breast cancer cells (MCF-7), and human prostate cancer cells (Du 145). Our results showed that hederagenin-coated magnetic nanoparticles (HMPs) may be useful as novel drug delivery systems; additionally, the nanoparticles responded well to external magnetic fields, which may allow for active drug targeting without concurrent system distribution.

Experimental

Synthesis and characterization of magnetic nanoparticles

The magnetic nanoparticles used in this study were synthesized from $FeCl_3 \cdot H_2O$ and $FeCl_2 \cdot 4H_2O$ (Duksan, Gyeonggi-do, Korea) using NH_4OH as a neutralizer. The solution was mixed well by 30 min of shaking, followed by vigorous stirring at room temperature with the slow addition of NH_4OH until a pH of 7 was reached. The addition of NH_4OH was stopped, the solution was continuously stirred for 2 h, and the magnetic nanoparticles were obtained. Electrolytes remaining in the solution were removed by washing with a mixture of acetone and methanol at a 1:1 ratio, followed by repeated washing with distilled water. Ethylene glycol (Extra Pure, Eg; Duksan) was used as the surfactant, followed by the addition of the magnetic nanoparticles.

Hederagenin-coated magnetic nanoparticle

HMP was produced using an emulsion technique [**20**]. The magnetic nanoparticles (5g) were added to 50 mL of distilled water. Subsequently, oleanolic acid (0.2 g) and dimethyl sulfoxide (50 mL) were added to the solution with constant stirring for 1 h at 25 °C. HMPs were separated from the electrolytes remaining in solution using a magnet and then washed. The density of the solution was 0.0801 g/mL when the magnetic nanoparticles precipitated. This indicated that the nanoparticles had been successfully loaded with hederagenin for stabilization under various physiological conditions. The physical and optical characteristics of the materials prepared were assessed via X-ray diffraction (XRD, D/mAX-200, Rigaku Denki, Tokyo, Japan), Fourier transform infrared spectroscopy (FT-IR, PerkinElmer, Waltham, MA, USA) and field emission scanning electron microscopy (FE-SEM, S-4300, Hitachi, Tokyo, Japan). In order to calculate the efficiency of the coating, the surface electrical properties of the different particles were analyzed by energy-dispersive X-ray spectroscopy.

Cell culture assay and cytotoxicity of HMPs

DU145 cancer cells were routinely cultured in RPMI-1640 medium, supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin solution at 37 °C in an atmosphere containing 5 % CO_2. SH-SY5Y, HeLa, U87, and MCF-7 cells were obtained from ATCC (Manassas, VA, USA) and cultured in 10 % fetal bovine serum in Dulbecco's minimum essential medium containing 0.5 % penicillin and streptomycin. Cell viability was assessed using a3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [**20**]. The cells were treated with the γ-Fe_2O_3 nanoparticles and HMP at various doses. After incubation for 24 h, the MTT solution was added and the cells were incubated at 37 °C for 3 h. The supernatant was then removed and 200 µL of dimethyl sulfoxide was added. The percentage of viable cells was determined by measuring the absorbance at 540 nm using a microplate reader.

Apoptosis of HMPs

Apoptotic cells were quantified by annexin V-FITC/propidium iodide (PI) double staining using an Annexin V-FITC apoptosis detection kit (BD Pharmingen, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, the cells (1×10^6 cells/mL) were collected, washed twice with PBS, and resuspended in binding buffer before the addition of annexin V-FITC and PI. Thereafter, samples were incubated in the dark for 15 min at room temperature and analyzed using a flow cytometer.

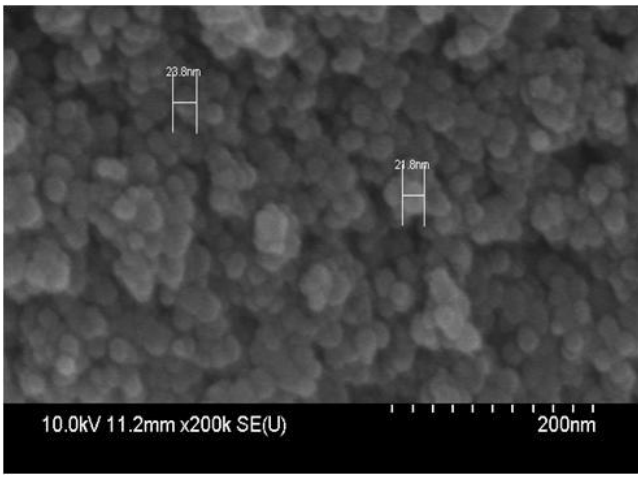

Fig. 1. Scanning electron micrograph image of Hederagenin–coated magnetic nanoparticles (HMPs).

Results and discussion

Characterization of magnetic nanoparticles and HMPs

FE-SEM was used to determine the size and morphology of the magnetic nanoparticles and HMPs. The SEM image in **Fig. 1** shows the spherical morphologies. Nanosphere sizes ranged from 21 to 45 nm, and the calculated average particle diameter was 32.5 nm, which was larger than that by SEM observation as shown in **Fig. 1**. The particle sizes of the samples were very consistent with the XRD results. **Fig. 2a** shows the XRD pattern of the magnetic nanoparticles prepared at a ratio of 1. All peaks were indexed to a cubic γ-Fe_2O_3 phase, which is consistent with the diffraction data reported in the 1997 JCPDS. The particle size of the nanoparticles was 32.5 nm according to the Debye–Scherrer formula. The XRD patterns of hederagenin showed no diffraction peaks on the HMP surface. HMPs and magnetic nanoparticles measured at room temperature are shown in **Fig 2b**. The saturation

magnetization value of HMPs was 60 emu/g, which was similar to the pattern of magnetic nanoparticles. This magnetic behavior indicated no hysteretic effect on the HMPs. The results confirmed that the super-paramagnetic properties of HMPs were mostly retained after encapsulation in hederagenin.

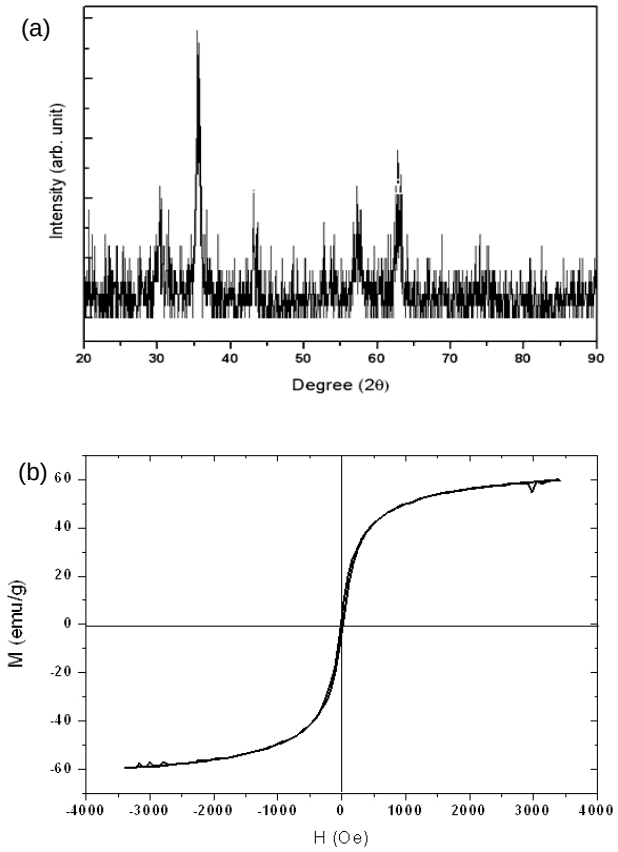

Fig. 2. XRD patterns (a) and vibrating sample magnetometer (b) of Hederagenin–coated magnetic nanoparticles (HMPs).

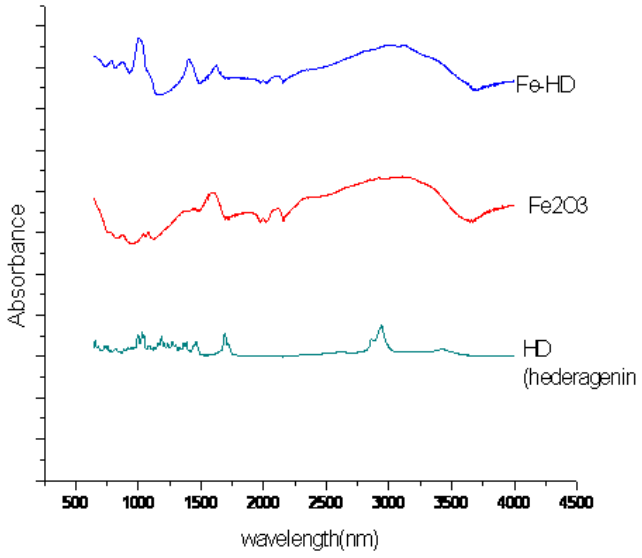

Fig. 3. FT-IR spectra of magnetic nanoparticles, pure hederagenin (HD), and hederagenin-coated magnetic nanoparticles (HMPs).

A PerkinElmer Spectrum 100 FT-IR Spectrometer with a diamond/ZnSe plate was used for analysis (**Fig. 3**). Spectra were recorded in the range of 600–4500 cm^{-1}. The C-H stretching band of HMP was observed at around 2970 cm^{-1} and a weaker peak at 1605 cm^{-1} corresponding to the stretching vibration band of C=C in hederagenin [**21**]. The peak at 1384 cm^{-1} was assigned to the in-plane bending vibration of the -OH and the peak at 1073 cm^{-1} was attributed to the stretching vibration of C-O [**22**].

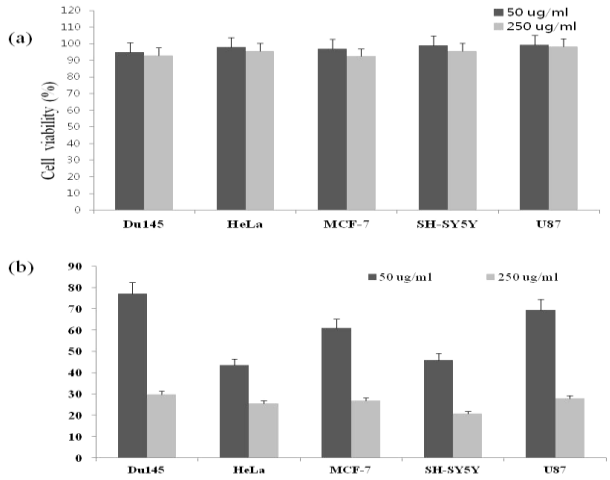

Fig. 4. Cell viability exposed to magnetic nanoparticles (a) and hederagenin-coated magnetic nanoparticles (HMPs) (b) against human brain cancer cell (SH-SY5Y), human cervical cancer cells (HeLa), human prostate cancer cells (DU145), breast cancer cells (MCF-7), and neuroblast cancer cells (U87). Cell viability was determined using an MTT assay.

Anticancer activity of HMPs

The cytotoxicities of the HMPs and magnetic nanoparticles were determined via an MTT test (**Fig. 4**). The magnetic nanoparticles showed no cytotoxicity against cancer cells, including SH-SY5Y, HeLa, DU145, MCF-7, and U87 cells at 50–250 μg/mL (**Fig. 4a**). These results demonstrate the biostability of the magnetic nanoparticles as drug carriers. However, the HMPs showed cytotoxicity at 23 % (DU145), at 54.04 % (SH-SY5Y), at 38.97 % (MCF-7), at 30.443 % (U87), and at 56.5 % (HeLa) at 50 μg/mL. At 250 μg/mL, the HMPs showed cytotoxicity at 70.2 % (DU145), at 79.154 % (SH-SY5Y), at 73.18 % (MCF-7), at 72.15 % (U87), and 74.5 % (HeLa) (**Fig. 4b**). Additionally, the cell viability of HMPs decreased from 23 % to 79.15 % in a dose-dependent manner. The decrease in cell viability with increasing HMP concentration was attributed to the increase in HMP uptake and corresponding increase in intracellular hederagenin concentration. In particular, the cytotoxicity of HMP in cancer cells inhibited cell proliferation. Thus, our results showed that the HMPs were effective in inhibiting cancer cell growth and tumorigenesis. It was previously reported that the cellular uptake of the nanoparticles was primarily mediated by a nonspecific endocytotic process [**23**]. The principal anti-tumor mechanism of hederagenin involves the association with an apoptosis signal [**24**]. Additionally, our results showed that HMP enhanced both the efficiency and therapeutic effects of the drug.

HMPs increased the number of apoptotic cells compared to the magnetic nanoparticles and pure

hederagenin treatment of breast cancer cells such as MCF-7 at 250 μg/mL. As shown in **Fig. 5**, HMPs showed at 79.6 % compared with only 1.196 % (magnetic nanoparticles) in MCF-7cell. The apoptosis induced by HMPs was 17.05 % higher than that caused by pure hederagenin. We found that HMP treatment reduced proliferation and increased apoptosis in breast cancer cells.

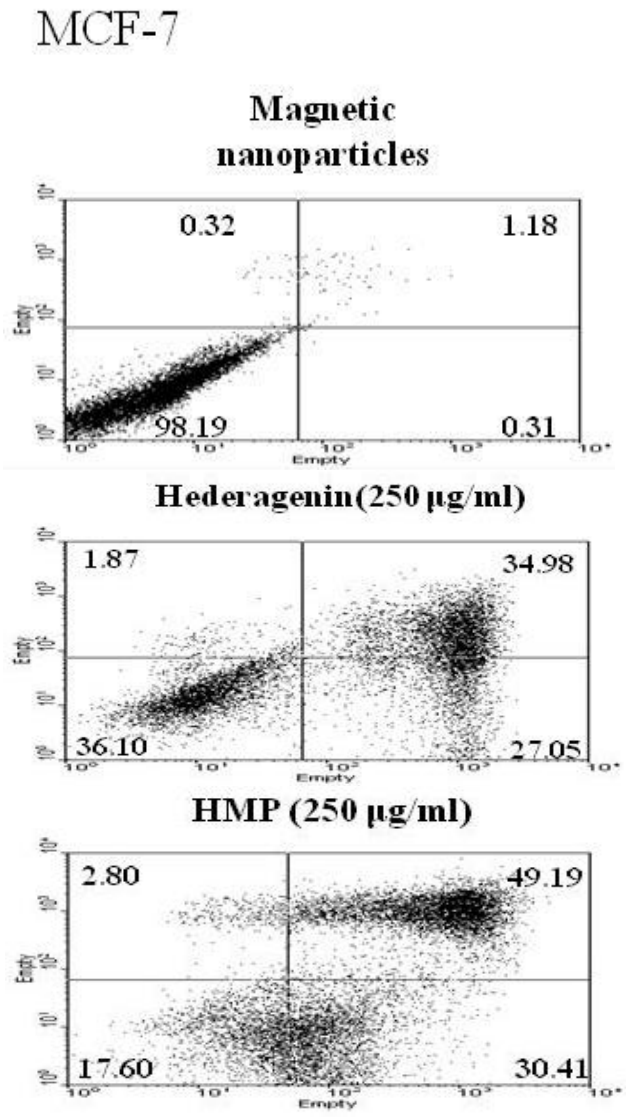

Fig. 5. Hederagenin–coated magnetic nanoparticles (HMPs) and γ-Fe_2O_3 nanoparticles induced apoptotic cell death in MCF-7 cell lines. Annexin V-FITC/PI staining analysis for apoptosis. Cells were treated with hederagenin, HMP, and γ-Fe_2O_3 nanoparticles (250 μg/mL). After treatment, the cells were stained with Annexin V-FITC/PI and subjected to flow cytometry analysis.

Conclusion

In this study, a new approach to the binding of HMPs was proposed and evaluated. The HMPs were spherical in shape and had an average diameter of 32.5 nm. Magnetic nanoparticles are biostable materials that can be used as drug carriers. However, at 250μg/mL, HMPs showed cytotoxicity at 70.2 % (DU145), at 79.154 % (SH-SY5Y), at 73.18 % (MCF-7), at 72.15 % (U87), and at 74.5 % (HeLa) (**Fig. 4b**). Additionally, HMPs decreased cell viability from 23 % to 79.15 % in a dose-dependent manner. The apoptosis index of the HMPs increased to 79.6 % compared to magnetic nanoparticle-treated cancer cells. This study demonstrated the potential efficacy of the biological applications of HMPs as anticancer agents. Additionally, the physical and biological characteristics of these functionalized HMPs will enable further chemical derivation, allowing for the exploitation of their magnetic properties to specifically target drugs to a tumor area using external magnetic fields. Thus, our results suggest that magnetic nanoparticles are biostable and that HMPs can be used for effective drug delivery.

Acknowledgements

This research was supported by the iPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), the High Value-added Food Technology Development Program (112123-3), and the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea and by Basic Science Research Program through the National Research Foundation of Korea (NRF) founded by the Ministry of Education (2014R1A1A2058817).

Author contributions

Conceived the plan: Jeung Hee An; Performed the experiments: Kwon Jai Lee and Jeung Hee An; Data analysis: Jae-Soo Shin, Dong-Hee Kim; Wrote the paper: Jeung Hee An. Authors have no competing financial interests.

Reference

1. Gozuacik D; Yagci-Acar HF; Akkoc Y; Kosar A; Dogan-Ekici AI; Ekici S.; *J Biomed Nanotechnol*. **2014**,*10*,1751. **DOI:** 10.1166/jbn.2014.1935
2. Wu Y; Wang Z; Liu G; Zeng X; Wang X; Gao Y; Jiang L; Shi X; Tao W; Huang L; Mei L; *J. Biomed. Nanotechnol*. **2015**, *11*, 1247. **DOI**: 10.1166/jbn.2015.2068
3. Anh Tran T; Krishnamoorthy K; Song YW; Cho SK; Kim SJ.; *ACS Appl. Mater. Interfaces*,**2014**, *6*, 2980. **DOI:** 10.1021/am405586d
4. Laurent S; Saei AA; Behzadi S; Panahifar A; Mahmoudi M; *Expert Opin Drug Deliv*.**2014**,*11*,1449. **DOI:** 10.1517/17425247.2014.924501
5. Lin L, Xiong L, Wen Y, Lei S, Deng X, Liu Z, Chen W, Miao X., *J. Biomed. Nanotechnol*, **2015**, *11*, 531. **DOI**: 10.1166/jbn.2015.2090
6. Yang K; Wan J; Zhang S; Tian B; Zhang Y; Liu Z; *Biomaterials* **2012**, *33*, 2206. **DOI:** 10.1016/j.biomaterials.2011.11.064
7. Babu A; Jeyasubramanian K; Gunasekaran P; Murugesan R;. *J. Biomed. Nanotechnol*. **2012**, *8*, 43. **DOI:** 10.1166/jbn.2012.1354
8. Lübbe AS; Alexiou C; Bergemann C.; *J. Surgical Research*, **2001**, *95*, 200. **DOI:** 10.1006/jsre.2000.6030
9. Martina MS; Wilhelm C; Lesieur S.; *Biomaterials* **2008**, *29*, 4137 . **DOI:** 10.1016/j.biomaterials.2008.07.011
10. Lee KJ; An JH; Chun JR; Chung KH; Park WY; Shin JS; Kim DH; Bahk YY; *J Biomed Nanotechnol*. **2013**, *9*, 1071. **DOI:** 10.1166/jbn.2013.1530
11. Lee KJ; An JH; Shin JS; Kim DH; *J Nanosci Nanotechnol*. **2010**, *10*, 6962.. **DOI:** 10.1166/jnn.2010.2985
12. Zablotskii V; Lunov O;, Gómez-Polo C; *J. Nanosci. Nanotechnol*. **2010**, *10*, 690 . **DOI:** 10.1166/jnn.2010.1806
13. Tietze R, Zaloga J, Unterweger H, Lyer S, Friedrich RP, Janko C, Pöttler M, Dürr S, Alexiou C. *Biochem Biophys Res Commun*. **2015**, pii: S0006-291X(15)30412-5. **DOI:** 10.1016/j.bbrc.2015.08.022.
14. Vincken, J. P.; Heng L; de Groot A.; Gruppen H.; *Phytochemistry*.**2007**, *68*, 275. **DOI:** 10.1016/j.phytochem.2006.10.008
15. Huang H. C.; Tsai W. J.; Liaw C. C.; Wu S. H.; Wu Y. C.; Kuo Y. H.; *Chem. Pharm. Bull.*, **2007**, *55*, 1412.

DOI: 10.1248/cpb.55.1412
16. Son I. H.; Park Y. H.; Lee S. I.; Yang H. D.; Moon H. I.; *Molecules*, **2007**, *12*, 1147.
DOI: 10.3390/12051147
17. Sparg S.G.; Light M.E.; Staden, J. van; *J. Ethnopharmacol*., **2004**, *94* , 219
DOI: 10.1016/j.jep.2004.05.016
18. Setzer,W.N.; Setzer ,M.C.; *Mini Rev. Med. Chem*.,**2003**, *3* , 540
DOI: 10.2174/1389557033487854
19. Wang J; Zhao XZ; Qi Q; Tao L; Zhao Q.; Mu R.; Gu HY.; Wang M.; Feng X.; Guo QL.; *Food Chem Toxicol*.**2009**,*47*,1716.
DOI: 10.1016/j.fct.2009.04.034
20. Lee K.J.; An JH; Shin JS; Kim DH; Kim C; Ozaki H; Koh JG; *Nanotechnology*, **2007**,*18*,465201.
DOI: 10.1088/0957-4484/18/46/465201
21. Feng J.; Chen Y.; Liu X.; Liu S.;*Food Chem.* **2015** ,*171*,272.
DOI: 10.1016/j.foodchem.2014.08.125
22. Wang C; Chao Z.; Sun W.; Wu X.;.; Ito Y. *J Liq Chromatogr Relat Technol*. **2014**, *37*, 2363.
DOI: 10.1080/10826076.2013.832297
23. Chawla J. S.; Amiji M. M.; *AAPS Pharmsci*.**2003**, *5*, E3.
DOI: 10.1208/ps050103
24. Lin, Y.; Kokontis, J.; Tang, F.; Godfrey, B.; Liao, S.; Lin, A.; Chen ,Y.; Xiang, J; *Mol. Cell Biol*. **2006**, *26*, 1908
DOI: 10.1128/MCB.26.5.1908-1916.2006

.

