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RESEARCH ARTICLE

Ginsenosides Extracted from Nanoscale Chinese White Ginseng Enhances Anticancer Effect

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Ginsenosides, the major chemical composition of Chinese white ginseng (*Panax ginseng* C. A. Meyer), can inhibit tumor, enhance body immune function, prevent neurodegeneration. In this paper, for the first time we reported that the amount of ginsenosides in the equivalent extraction of the nanoscale Chinese white ginseng particles (NWGP) was 2.5 times more than that of microscale Chinese white ginseng particles (WGP). And the extractions from NWGP (1000 μg/ml) reached a high tumor inhibition of 64% exposed to human lung carcinoma cells (A549) and 74% exposed to human cervical cancer cells (Hela) after 72 h. Our work shows that the nanoscale Chinese WGP greatly improves the bioavailability of ginsenosides.

Keywords: Ginsenosides, Nanoscale Chinese White Ginseng, Anticancer, *In Vitro*.

1. INTRODUCTION

Chinese white ginseng (*Panax ginseng* C. A. Meyer) is one of the precious herbs in traditional Chinese medicine (TCM) as a tonic, prophylactic agent in the whole world, particularly in China, Korea, and Japan.[1] In the past decade, promising advances were achieved in understanding the chemistry,[2] pharmacology,[3] and structure-function relationship of Chinese white ginseng.[4] Ginsenosides are the main ingredients of Chinese white ginseng and have lots of pharmacological activities, such as anti-tumor,[5] antioxidation,[6] immunomodulation.[7] However, ginsenosides are expensive and not suitable for long-term use. Therefore, it is necessary to improve the utilization of the ginsenosides in the Chinese white ginseng.

Nanomaterials have displayed potential applications[8] in the fields ranging from drug delivery[9] to imaging.[10] There have been some studies on the use of nanotechnology in TCM,[11] such as some TCM raw materials were directly crushed into nanometer size by machines,[12] some active ingredients of TCM materials and nanocarriers composed the drug-nanocarrier systems.[13] The drug efficacy is improved using the above mentioned methods,[14] because the nanoparticles have larger specific surface areas, stronger solvent penetration and thus higher extraction yield. It was reported that the ginseng powders about 8 micrometers was prepared by the ultra-fine grinding processing.[15] However, to the best of our knowledge, the anti-cancer application of the nanoscale Chinese white ginseng was never reported before.

In this study, we compared the amount and the anti-cancer property of ginsenosides in the microscale Chinese white ginseng particles (WGP) and the nanoscale Chinese white ginseng particles (NWGP). We found that the amount of ginsenosides in the equivalent extraction of the NWGP samples were more than that in the microscale WGP samples, and the NWGP extractions also had a stronger tumor inhibition effect than that in the microscale WGP extractions due to the facilitation of the larger specific surface area of the NWGP.

2. EXPERIMENTAL DETAILS

2.1. Materials

Chinese white ginsengs (*Panax ginseng* C. A. Meyer) were cultivated and identified (by Pro. Bao) in Jilin Agricultural University (Changchun, China). Ginsenosides Rg1, Re, Rb1, and Rd were purchased from National Institutes for Food and Drug Control (Beijing, China).

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2.2. Preparation and Characterization of WGP

As a starting material, Chinese white ginseng samples with the size of 100 mesh (150 ± 6.6 μm) was used. 2.89 g WGP mixed with agate balls with 10, 6 mm (Φ10 mm:Φ6 mm = 50:10) of diameter were sealed in a polytetrafluoroethylene installation with agate balls-to-WGP weight ratio of 10:1 and rotated at speed of 600 rpm. The NWGP was obtained following the three-step procedure for 30 h, clockwise rotation-stillness-anticlockwise rotation with each step kept for 30 min. The milling was performed with Type QM-3SP04 planetary ball bill (China).

The morphologies and sizes of WGP and NWGP were characterized by an S-4800 field emission scanning electron microscopy (SEM, Hitachi, Japan).

2.3. Ginsenosides Extraction

2.3.1. Extraction for FTIR and Anti-Tumor In Vitro Tests

Ultrasound-assisted extraction was performed in an ultrasonic cleaner with the output power of 100 W and the frequency of 40 kHz. 2.00 g WGP and NWGP were placed into two 100 ml conical flasks respectively, and 60 ml of 70% (v/v) ethanol-water were added. The mixtures were filtrated, and the extractions were transferred into a 200 ml volumetric flask after 30 min in an ultrasonic water bath, then removed the solvent using an evaporator. The samples used for Fourier transform infrared spectrometer (PerKin-Elmer) and anti-tumor *in vitro* tests were dried by freeze-drying.

2.3.2. HPLC Sample Preparation

0.50 g WGP and 0.50 g NWGP samples were extracted with 70% ethanol–water in a Soxhlet extractor for 8 h at 85 °C, respectively. The extracts were concentrated in vacuum, transferred into a 10 ml volumetric flask, and then diluted to the desired volume with methanol (HPLC grade, Duksan Pure Chemical Co., Ltd.). Two samples were prepared in parallel to test the variations, and all experiments were performed at least 3 times. The solutions were stored at 4 °C until HPLC analysis.

2.4. Chromatographic Conditions

Chromatographic analysis was performed using a Waters 600E HPLC instrument (Milford, MA) with a quaternary pump, an automatic injector, and a UV detector (Model 2487). Chromatographic separation was carried out on an Atlantis dC18 column (5 μm, 4.6×150 mm, Waters Crop.) at a column temperature of 30 °C. The detection wavelength was set to 203 nm. The mobile phase consisted of acetonitrile (A) and water (B) using a gradient elution of 22–30% A at 0–20 min, 30–33% A at 20–25 min, 33–37% A at 25–35 min, 37–50% A at 35–40 min, 50–22% A at 40–50 min. The flow rate was kept at 1 ml/min, and the injected sample volume was set at 10 μl.

2.5. Experiments of Anti-Tumor *In Vitro*

2.5.1. Cell Culture

Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with L-alanyl-L-glutamine (2 mM), antibiotics (100 U/ml, penicillin-streptomycin), and 10% fetal bovine serum (FBS). Cells were maintained in a humidified atmosphere at 37 °C and 5% CO_2.

2.5.2. Anti-Tumor In Vitro

We employed the classic CCK-8 viability assay to evaluate and compare the anticancer effect of WGP and NWGP. The human lung carcinoma epithelial-like cell lines (A549) were plated in the 96-well plates (3×10^3 cells per well) and incubated for 24 h, and human cervical cancer cell lines (Hela) were plated in the 96-well plates (5×10^3 cells per well) and incubated for 24 h. WGP and NWGP were introduced separately to cells with different test concentrations (50, 100, 200, 500, 1000 μg/ml) in the culture medium. Cells cultured in the medium without ginseng were taken as the control. After 24, 48, and 72 h incubation, the cells were washed with phosphate buffer solution (PBS) three times. 100 μl DMEM containing CCK-8 (10%, v/v, Dojindo Molecular Technologies, Inc.)were added to each well. After 2 h incubation at 37 °C, the absorbance at 450 nm of each well was measured using a microplate reader (TECAN Infinite M200, Austria).

2.6. Statistical Analysis

All data are expressed as the mean with the standard deviation (mean $\pm$ SD). A one-way analysis of variance (ANOVA) was employed to the results from studies on cell viability. The unpaired Student's t test was applied to identify significant differences between the groups treated with WGP and NWGP. $P < 0.05$ is considered to be a significant difference. * denotes a statistical significance ($^* < 0.05$ and $^{**} < 0.01$) of NWGP versus WGP.

3. RESULTS AND DISCUSSION

In this study, NWGP was prepared by dry milling WGP (100 mesh, 150 ± 6.6 μm) in a planetary ball bill as described in the experimental section. We found that the nanoscale WGP have a lighter color observed with the naked eyes and feel smoother in hands compared

to the microscale WGP. The SEM image (Fig. 1(a)) displayed that the WGP samples had a large tangled agglomeration, crude surfaces, and the size ranging from 3 to 8 μm. In contrast, Figure 1(b)–(c) showed that the NWGP had the loose fine particles with irregular shapes and smaller sizes in the range of 500–600 nm. Obviously, the NWGP had larger specific surface areas than the microsized WGP.

In our experiment, ginsenosides extractions from WGP and NWGP were collected and analyzed by FTIR spectrometer. The infrared spectra (red) in Figure 2 showed the functional groups of ginsenosides in NWGP. For instance, the strongest peak at 3402 cm^{-1} was belonged to the stretching vibration of O–H groups, the peak at 2930 cm^{-1} was assigned as the stretching vibration of C–H in methyl and methylene groups,[16] the peak of symmetric and asymmetric COO- stretching vibration was focused on 1640 cm^{-1} and 1406 cm^{-1},[17] respectively. 1051 cm^{-1} was assigned as the bending vibration of C-C-O or C-C-OH in starch.[18] The infrared spectra characteristics of ginsenosides in WGP were similar to those in NWGP, although the peak intensity of ginsenosides in the WGP samples and the NWGP samples was different. Therefore, the chemical structures of ginsenosides didn't change from microscale WGP to NWGP.

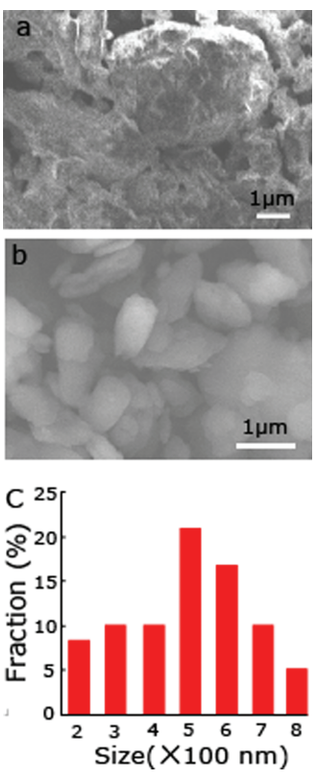

Fig. 1. SEM images of Chinese white ginseng samples in microsize (a) and nanosize (b), (c) A histogram of the nanosized WGP by measuring the dimensional sizes.

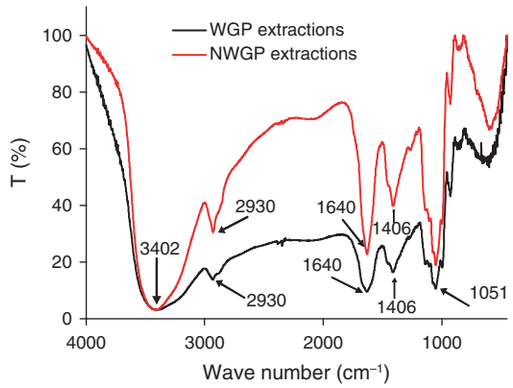

Fig. 2. FTIR spectra of WGP extractions (black) and NWGP extractions (red) with KBr disc in the ranges from 4000 to 400 cm^{-1}.

It is believed that the major bioactive compounds in Chinese white ginseng are ginsenosides.[19] The typical HPLC-UV chromatograms of ginsenosides extracts from WGP and NWGP were shown in Figures 3(b) and (c), respectively. Based on the component analysis and the comparison between individual and mixed ginsenoside, the structures were the same due to the existence of the same retention time, and Table I indicated that there were great difference in the content percentage of ginsenosides from WGP and NWGP samples. Ginsenosides Rb1, Rd, Rg1, and Re were four major ginsenosides in white ginseng, accounting for more than 70% of the total ginsenoside contents.[20] Due to the distinct variation in contents of ginsenosides in the different Chinese white ginseng samples, four methanol stock solutions of standards (Rg1, Re, Rb1, Rd) were prepared and diluted with methanol solution to appropriate concentration for the establishment of

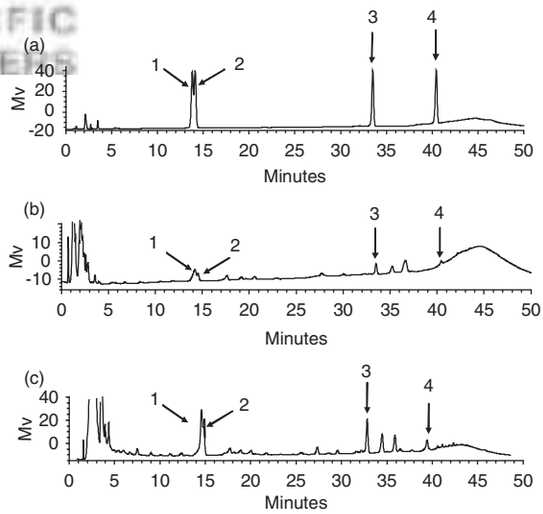

Fig. 3. Typical HPLC-UV chromatograms at 203 nm of Chinese white ginseng: (a) mixture standards of Chinese white ginseng, (b) WGP, (c) NWGP. Ginsenoside peaks: (1) Rg1, (2) Re, (3) Rb1, (4) Rd. The concentrations of ginsenoside standards were all 0.5 mg/ml.

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Table I. The contents of Rb1 and Rd in WGP and NWGP by the HPLC calibration curve.

Gin senoside	RT (min)	Calibration curve[a]	Correlation coefficient (r^2)	Extraction percentage (mg/g dry WG) WGP	NWGP
Rg1	13.81	$A = 2 \times 10^6 c$-118076	0.9980	2.370	6.312
Re	14.11	$A = 2 \times 10^6 c$-69464	0.9965	0.972	2.978
Rb1	33.51	$A = 2 \times 10^6 c$-113139	0.9997	1.500	3.868
Rd	40.44	$A = 2 \times 10^6 c$-82154	0.9992	0.919	2.638

[a] A = Peak Area, c = Concentrations (μg/ml).

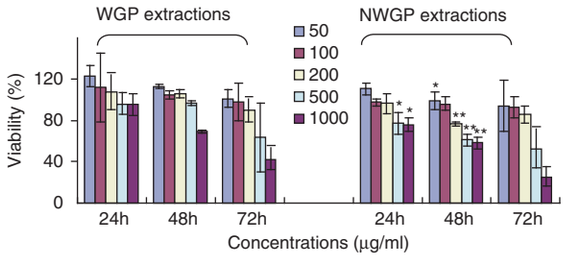

Fig. 5. The viability of Hela cells after exposed to the WGP extractions and the NWGP extractions (50, 100, 200, 500, 1000 μg/ml) for 24 h, 48 h, 72 h.

calibration curves. Five concentrations (0.02–0.5 mg/ml) of four ginsenosides solutions were injected in triplicate, and then the calibration curves were constructed by plotting the peak areas against the concentration of each analyte. Table I showed that the content percentage of four ginsenosides in NWGP was 2.5 times more than that in WGP according to the HPLC-UV chromatograms. Though the baseline shifted occasionally, there was little influence on the results. So the extraction percentage of ginsenosides from NWGP was greatly increased compared with that from WGP.

The cell viability was investigated to estimate the anticancer property of Chinese white ginseng samples quantitatively by Cell Counting Kit-8 (CCK-8) assay.[21] Briefly, a certain volume of solution containing tetrazolium salt (available in the CCK-8 Kit from Dojindo Laboratories, Japan) was used. The mitochondria's ability to reduce a tetrazolium salt to a formazan dye was used to assess mitochondrial dehydrogenase activity. Traditionally, we always used MTT or MTS[22] test to evaluate the cytotoxicity. However, the CCK-8 solution was more convenient and the absorbance of the CCK-8 assay solution was higher than that of MTT or MTS. Therefore, we chose the CCK-8 solution to test the cell viability. The tumor inhibition or cytotoxicity was the inverse of cell viability (% of control). The cell viability was expressed as the percentage of $(OD_{\text{test}}–OD_{\text{blank}})/(OD_{\text{control}}–OD_{\text{blank}})$, where OD_{test} was the optical density of the cells exposed to ginseng samples, OD_{control} was the optical density of the control sample and OD_{blank} was the optical density of the wells without A549 cells or Hela cells. Figure 4 indicated a dose-dependent and time-dependent cytotoxicity in terms of CCK-8 reduction in A549 cells. At higher concentrations and longer time, the viability loss was observed in the cells treated with ginsenosides both in WGP extractions and NWGP extractions. The influence of WGP extractions on the tumor inhibition of A549 cells was tiny in 24 h. Even at the highest concentration of 1000 μg/ml, less than 15% of the cell tumor inhibition remained. However, the tumor inhibition of NWGP in A549 cells for 24 h, 48 h at 1000 μg/ml, near 30% and 35% of the cell inhibition remained. So NWGP extractions enhanced the anti-tumor effects. Similar results were obtained from 72 h exposure, the highest tumor inhibition up to 62%.

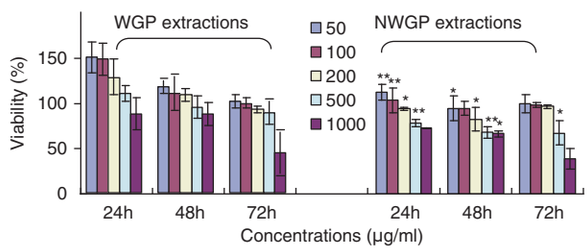

Fig. 4. The viability of A549 cells after exposed to the WGP extractions and the NWGP extractions (50, 100, 200, 500, 1000 μg/ml) for 24 h, 48 h, 72 h.

Likewise, Figure 5 displayed a dose-dependent and time-dependent cytotoxicity in terms of CCK-8 reduction in Hela cells. The influence of WGP extractions on the tumor inhibition of Hela cells was also tiny in 24 h. Even at the highest concentration of 1000 μg/ml, only 5% of the cell tumor inhibition remained. However, the tumor inhibition of NWGP in Hela cells for 24 h, 48 h at 1000 μg/ml, near 25% and 40% of the cell inhibition remained. The highest tumor inhibition up to 74% at 1000 μg/ml exposure 72 h in Hela cells. These results collectively indicated that NWGP extractions enhanced the anti-tumor effects to a great extent.

In short, the amount of ginsenosides in the NWGP extractions was more than 2.5 times compared with that in the WGP extractions according to the HPLC-UV chromatograms, and the NWGP extractions enhanced the anti-tumor effects compared with the WGP extractions in A549 cells and Hela cells, especially exposed to A549 cells in a short time. We proposed that the larger specific surface area of NWGP facilitated the extraction of ginsenosides. That is, the NWGP extractions enhanced the anti-tumor effects due to a higher bioavailability of the ginsenosides in the NWGP compared to that in the microscale WGP.

4. CONCLUSIONS

In summary, we successfully prepared nanoscale Chinese white ginseng with 500–600 nm by dry milling in a

planetary ball bill, and the amount of ginsenosides in the equivalent extraction of the NWGP was 2.5 times more than that of microscale WGP. Furthermore, the NWGP extractions reached a higher tumor inhibition exposed to A549 cells and Hela cells compared with the WGP extractions. Our work shows that the nanoscale Chinese white ginseng greatly improves the bioavailability of ginsenosides, which are essential for economizing the herb materials and improving officinal value.

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References and Notes

1. (a) S. Zhang, R. Chen, H. Wu, and C. Wang, *J. Pharm. Biomed. Anal.* 41, 57 (**2006**); (b) T. K. O. O. Yun, *Ann. N.Y. Acad. Sci.* 889, 157 (**1999**); (c) K. Choi, *Acta. Pharm. Sin.* 29, 1109 (**2008**); (d) Y. Sasaki, K. Komatsu, and S. Nagumo, *Bio. Pharmac. Bul.* 31, 1806 (**2008**).
2. N. Fuzzati, *J. Chromatogr. B.* 812, 119 (**2004**).
3. (a) C. L. Broadhurst, M. M. Polansky, and R. A. Anderson, *J. Agric. Food. Chem.* 48, 849 (**2000**); (b) L. Wang, and W. S. Kisaalita, *J. Neurosci. Methods* 2, 274 (**2010**); (c) L. Wang, Q. M. Liu, B. H. Sung, D. S. An, H. G. Lee, S. G. Kim, S. C. Kim, S. T. Lee, and W. T. Im, *J. Biotechnol.* 2, 125 (**2011**).
4. (a) S. Shibata, *J. Korean Med. Sci.* 16, S28 (**2001**); (b) E. A. Bae, M. J. Han, M. K. Choo, S. Y. Park, and D. H. Kim, *Bio. Pharm. Bull.* 25, 58 (**2002**).
5. (a) C. Chen, W. Chiou, and J. Zhang, *Acta. Pharmacol. Sin.* 29, 1103 (**2008**); (b) A. B. Fishbein, C. Z. Wang, X. L. Li, S. R. Mehendale, S. Sun, H. H. Aung, and C. S. Yuan, *Arch. Pharm. Res.* 32, 505 (**2009**); (c) V. K. W. Wong, S. S. F. Cheung, T. Li, Z. H. Jiang, J. R. Wang, H. Dong, X. Q. Yi, H. Zhou, and L. Liu. *J. Cell. Biochem.* 111, 899 (**2010**); (d) D. C. W. Lee, and A. S. Y. Lau, *Molecules* 16, 2802 (**2011**).
6. (a) C. Y. O. Chen, J. D. Ribaya-Mercado, D. L. McKay, E. Croom, and J. B. Blumberg, *Food Chem.* 119, 445 (**2010**); (b) K. Norajit, K. M. Kim, and G. H. Ryu, *J. Food Eng.* 98, 377 (**2010**).
7. H. Wang, J. K. Actor, J. Indrigo, M. Olsen, and A. Dasgupta, *Clin. Chim. Acta.* 327, 123 (**2003**).
8. M. R. -M. I. Imaz, W. J. Saletra, L. Garcıa-Fernaındez, F. Garcıa, D. Ruiz-Molina, J. Hernando, V. Puntes, and D. Maspoch, *Chem. Commun.* 46, 4737 (**2010**).
9. C. R. Martin, and P. Kohli, *Nat. Rev. Drug Discovery* 2, 29 (**2003**).
10. P. K. Jain, X. Huang, I. H. El-Sayed, and M. A. El-Sayed, *Acc. Chem. Res.* 41, 1578 (**2008**).
11. K. Leonard, B. Ahmmad, H. Okamura, and J. Kurawaki, *Colloids Surf. B.* 82, 391 (**2011**).
12. (a) Y. Su, Z. Fu, J. Zhang, W. Wang, H. Wang, Y. Wang, and Q. Zhang, *Powder Technol.* 184, 114 (**2008**); (b) H. W. Wen, W. C. Li, R. J. Chung, S. Y. Yin, T. H. Chou, P. C. Hsieh, P. H. Wang, and I. Lin, *J. Nanosci. Nanotechnol.* 7, 4108 (**2009**).
13. (a) F. Xiong, H. Chen, X. Chang, Y. Yang, H. Xu, and X. Yang, *IEEE* 10, 4966 (**2006**); (b) X. Song, L. Zang, and S. Hu. *Vaccine* 27, 2306 (**2009**).
14. (a) M. Murakami, H. Cabral, Y. Matsumoto, S. Wu, M. R. Kano, T. Yamori, N. Nishiyama, and K. Kataoka, *Sci. Trans. Med.* 3, 642 (**2011**); (b) Z. Zhang, W. Cao, H. Jin, J. F. Lovell, M. Yang, L. Ding, J. Chen, I. Corbin, Q. Luo, and G. Zheng, *Angew. Chem. Int. Ed.* 48, 9171 (**2009**).
15. Y. Su, Z. Y. Fu, W. M. Wang, H. Wang, Y. C. Wang, J. Y. Zhang, and P. Ma, *Key. Eng. Mater.* 330, 215 (**2007**).
16. R. Li, W. Dong, H. He, H. Yan, X. Jiang, and J. Gong, *Cry. Res. Technol.* 4, 377 (**2012**).
17. G. Lu, Q. Zhou, S. Sun, K. S. Leung, H. Zhang, and Z. Zhao, *J. Mol. Struct.* 883, 91 (**2008**).
18. D. Liu, Y. G. Li, H. Xu, S. Q. Sun, and Z. T. Wang, *J. Mol. Struct.* 883, 228 (**2008**).
19. B. S. Sun, L. J. Gu, Z. M. Fang, C. Wang, Z. Wang, M. R. Lee, Z. Li, J. J. Li, and C. K. Sung, *J. Pharm. Biomed. Anal.* 50, 15 (**2009**).
20. H. Y. Kim and K. Kim, *J. Agric. Food Chem.* 55, 2816 (**2007**).
21. (a) J. J. Yin, F. Lao, P. P. Fu, W. G. Wamer, Y. Zhao, P. C. Wang, Y. Qiu, B. Sun, G. Xing, and J. Dong, *Biomaterials* 30, 611 (**2009**); (b) Y. Chang, S. T. Yang, J. H. Liu, E. Dong, Y. Wang, A. Cao, Y. Liu, and H. Wang, *Toxicol. Lett.* 200, 201 (**2010**).
22. S. Sun, L. W. Qi, G. J. Du, S. R. Mehendale, C. Z. Wang, and C. S. Yuan, *Food Chem.* 125, 1299 (**2011**).

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