Research Article

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Synthesis of PEITC-loaded gold nanoparticles and evaluation of the hepatoprotective effect on CCl_4-induced damage through Nrf2 pathway

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Abstract

Objectives: The purpose of the study was to prepare an effective and new drug delivery system for enhancing the stability of Phenethyl isothiocyanate (PEITC), and its hepatoprotective effect in the carbon tetrachloride (CCl_4)-induced damage in hepatocellular carcinoma G2 (HepG2) cell line via nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway.

Methods: Gold nanoparticles were synthesized and then characterized by XRD, SEM, SEM-EDX analysis, hydrodynamic diameter and zeta potential measurements. 1.0024 mM PEITC, a naturally occurring isothiocyanate, an active ingredient was loaded onto the characterized AuNPs. The cytotoxicity test of PEITC-AuNP and effects on ALT, AST, Nrf2 levels and total antioxidant capacity (TAC) of CCl_4-induced HepG2 cells were investigated.

Results: PEITC-AuNPs and PEITC decreased ALT and AST levels ($p<0.05$). This reduction was greater with PEITC-AuNPs. PEITC-AuNPs increased Nrf2 level but it was nonsignificantly ($p>0.05$). PEITC didn't increase the Nrf2 level in CCl_4-induced HepG2 cells. TAC of both PEITC-AuNPs and PEITC administration increased significantly compared with CCl_4 group ($p<0.05$). But PEITC-AuNPs enhanced the TAC level higher than PEITC significantly ($p<0.05$).

Conclusions: PEITC-AuNPs were more effective than PEITC which resulted in more hepatoprotective and antioxidant effects via Nrf2 activation against CCl_4-induced liver injury in HepG2 cells.

Keywords: CCl_4; gold nanoparticle; HepG2 cell; Nrf2; phenethyl isothiocyanate.

Introduction

Two million people a year die almost from liver diseases globally [1]. An increase in smoking, alcohol consumption and environmental pollution causes an enhancement incidence of liver disease [2]. The liver plays a critical role in the metabolism of xenobiotics, such as CCl_4 [3]. CCl_4 exposure leads to the hepatotoxicity through overproduction of free radicals such as trichloromethyl radical and its derivative, trichloromethyl peroxy radical. Thus, ROS elimination is crucial in the treatment and prevention of hepatic diseases [4]. CCl_4 is often-used to induce the acute liver injury model to study hepatoprotective agents [5]. Nrf2, a transcription factor, regulates antioxidant systems. Under normal conditions, Nrf2 and its inhibitor (INrf2) are bound. When oxidative stress occures, it separates and binds with antioxidant response elements (ARE) in the nucleus and induces lots of antioxidant and phase II detoxifying enzymes [6]. It has been reported that Nrf2 activation protected against CCl_4-induced liver damage [7]. Therefore, Nrf2 signalling pathway may be an effective target for protection and treatment of liver diseases.

In recent years, natural agents have been investigated for their hepatoprotective effect. Because natural agents reduce side effects compared to synthetic ones. Numerous drugs are utilized in liver disease treatment, but plenty of them have deficits such as poor target organ selectivity,

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lower tissue distribution, and *in vivo* instability. Furthermore, drug-induced toxicity severely limits the drug administration for liver diseases [8]. HepG2 cells are regarded as a sensitive model for investigating *in vitro* various metabolism and hepatotoxicity. It is also used to determine the hepatoprotective activity of natural products and nanoparticles [9, 10].

PEITC is an isothiocyanate in Cruciferous plants and is produced by hydrolysis of gluconasturtiin with myrosinase. It was demonstrated that PEITC suppressed oxidative stress and has a cytoprotective effect by activation of Nrf2 [11]. Wang et al. showed that PEITC protected against acute liver disease [12]. Although studies demonstrate the benefit of PEITC against diseases especially cancer, PEITC's clinical usage is still restricted because of its low solubility, and stability. Nano-delivery technology was used to the reduction of these disadvantages. Nanotechnology has a significant effect on the improvement of more efficient diagnostic and therapeutic methods [13]. In addition, it is used in many fields of biomedical science, and nano-targeted drug carrier systems in the last years [14]. The drug carrier systems mainly include liposomes, dendrimers, polymeric materials and metallic nanoparticles. AuNPs are an ideal drug delivery system due to their bio-inert, non-toxic and easy preparation properties [15]. Additionally, AuNPs have a surface that is easily functionalized with active targeting moieties and leads to obtaining multiple targeting agents [16]. Therefore, we aimed to develop the synthesis and characterization of PEITC loaded-gold nanoparticle and to evaluate its hepatoprotective effect on CCl_4^-induced liver injury in HepG2 cells through activation of Nrf2 signalling pathway.

Materials and methods

Synthesis and characterization of PEITC-loaded gold nanoparticles (PEITC-AuNPs)

AuNPs were synthesized by the seed growth method [17]. For the preparation of 3.5 ± 0.7 nm gold seed, 0.6 mL of 0.1 mol/L $NaBH_4$ solution was added to 20 mL of an aqueous solution (2.5×10^{-4} mol/L $HAuCl_4$ and 2.5×10^{-4} M trisodium citrate). The colour of the solution turning pink indicates particle formation. For the preparation of the nanoparticle, 7.5 mL of growth solution (2.5 × 10–4 mol/L with Cetyl trimethyl ammonium bromide) was taken and 0.1 mol/L 0.05 mL of ascorbic acid was added to it. Then 2.5 mL of seed solution was mixed. After 10 min, the colour of the solution changed to a wine colour.

For loading PEITC on AuNPs, 3.8 µL of PEITC was dissolved in 5 mL of DMSO and 0.025 g of AuNPs were dispersed in 6 mL of ultrapure water, and mixed in a magnetic stirrer at 200 rpm for 24 h.

The synthesized AuNPs were characterized using particle size distribution and zeta potential (Malvern Zeta-Sizer Nano series Nano-ZS), X-ray Diffractometer (Miniflex 600), SEM (Scanning electron Microscope-Digital LEO-EVO 40), and EDX (energy dispersion X-ray analysis – Bruker 125 eV) analyses.

To determine the loaded PEITC quantity into the prepared nanoparticle at the end of the loading process, a serial calibration solution with a PEITC concentration of 0.1, 0.25, 0.5, 1.0, and 5.0 mg/L, respectively, was prepared. The measurement was taken in a UV-Vis spectrophotometer at 305 nm.

Cell culture and cytotoxicity assay

HepG2 cell line was obtained from the American Type Culture Collection (ATCC, NY, USA). The cells were cultured in MEM medium supplemented with 1% (v/v) penicillin-streptomycin, 10% (v/v) heat-inactivated FBS, L-glutamine, and sodium pyruvate. Cells were maintained in a 5% CO_2 humidified incubator at 37 °C.

For the administration doses, PEITC-AuNPs were diluted with MEM cell culture medium to prepare various concentrations of 0.125, 0.25, 0.5, 1, 2, 4, 8, 10, and 20 nM HepG2 cells were treated with PEITC-AuNP administration doses for 24, 48, and 72 h at 37 °C with 5% CO_2. When HepG2 cells reached the 80% confluency, they were exposed to CCl_4 at various concentrations of 0.25, 0.5, 1, and 2% (v/v) for 2 h at 37 °C with 5% CO_2.

To evaluate the cytotoxicity induced by CCl_4 and PEITC-AuNPs on HepG2 cells, the MTT assay was performed [18]. 10^4 cells/well were plated in 96 well-microplates. The cells were treated with various concentrations of CCl_4 for 2 h and PEITC-AuNPs for 24, 48, and 72 h at 37 °C and 5% CO_2. Following the incubation period, 5 mg/mL concentration of MTT solution was added to wells and incubated for another 4 h. Then, cell culture media was removed and DMSO was added to solubilize the produced formazan, and absorbance was determined spectrophotometrically by using Biotek Synergy H1 multimode microplate reader at 570 nm.

Determination of ALT, AST, Nrf2, and total antioxidant capacity levels

After CCl_4 and PEITC-AuNP administration, ALT and AST levels were measured in the HepG2 cell culture supernatant by Auto Analyser (Beckman Coulter AU5800). Nrf2 level in HepG2 cell nuclear extract was measured with the Human Nrf2 Transcription Factor ELISA Kit (Elabscience, USA). Total antioxidant capacity in HepG2 cell cytosolic extract was measured with the Total Antioxidant Status Assay Kit (Rel Assay Diagnostics, Turkey).

Statistical analysis

For the analysis of the biochemical measurements data, the IBM SPSS Statistics 22.0 for Windows package program was used. The non-parametric Kruskal-Wallis and Mann-Whitney U tests were used. All results are presented as mean ± standard error (SE), and $p<0.05$ was accepted as statistically significant.

Results

Evaluation of synthesis and characterization of PEITC-AuNP

XRD analysis (X-ray diffractometry)

XRD analysis was performed to determine the phase analysis and crystal size of the synthesized AuNPs. The resulting XRD diffraction pattern is given in Supplementary material. In addition, the crystal size of the synthesized nanoparticle was calculated as 5.65 nm using the Scherer equation (1). In the equation, D; mean crystal size in nanometres, K; crystal form factor (0.94), λ; The wavelength of the X-ray beam (0.1548 nm for Cu Kα radiation), β; the width of the maximum scattering peak at half the height (FWHM), θ; represents the peak point (Bragg angle). XRD analysis was performed at a scanning range of 10–80° and a scanning degree of 2° min^{-1}.

$$D = \frac{K \times \lambda}{\beta \times \cos\theta} \quad (1)$$

It was determined that the synthesized nanoparticle gave peaks belonging to the AuNPs corresponding to the Miller indices of 111, 200, 220 and 311.

SEM analysis (scanning electron microscopy)

SEM analysis was performed to determine the nanocrystalline structures and surface morphologies of the synthesized AuNPs using a Digital LEO-EVO 40 device. In Supplementary material, it is seen that the synthesized nanoparticle has regular spherical particles and no agglomeration is observed.

Determination of zeta potentials and hydrodynamic diameters

The pH-dependent surface charges and isoelectric point zeta potentials of the synthesized AuNPs were determined. The results obtained were plotted against pH vs. zeta potential (mV), and the point where the curve cuts the x-axis was determined as the isoelectric point of the photocatalysts (see Supplementary Material). The pH values of the isoelectric points of AuNPs were determined as 7.54. Zeta potential values of AuNPs are higher than ± 30 mV at acidic or basic pH values. This result indicates that the nanocomposite material has good dispersion in aqueous media. The dynamic laser light scattering method (DLS) was used to determine the dispersed nanoparticle sizes and the translational diffusion coefficient (D). Therefore, it provides the hydrodynamic diameter of the substances in a solution by using the Stokes-Einstein equation.

$$d(H) = kT/3\pi\eta D \quad (2)$$

d (H): hydrodynamic diameter, k: boltzmann constant, T: temperature, η: viscosity, D: cyclic diffusion coefficient.

To determine the of the d (H) of the AuNPs was measured with a Zeta Sizer device. The results were given in Table 1 and Supplementary Material.

Loading PEITC on AuNPs

After the loading procedure, PEITC concentration in the drug carrier system (PEITC-AuNPs) measured in a UV-Vis spectrophotometer and its concentration was determined as 1.0024 mM. The calibration graph of PEITC is shown in Supplementary Material.

Cytotoxic activity of PEITC-AuNP on HepG2 cells

To determine the pretreatment dose of CCl_4, HepG2 cells were exposed to various increasing concentrations of CCl_4 (0.25, 0.5, 1, and 2% (v/v)). MTT cytotoxicity test resulted in the IC_{50} value being calculated as 1.8%. For the subsequent experiments pretreatment dose of CCl_4 was determined to be 1.5%. The change in the cell viability was found in a dose-dependent manner in 24 h incubation. 20 nM sublethal dose of PEITC-AuNPs for 24 h was selected as the administration dose for subsequent experiments (Figure 1A, B).

Effect of PEITC-AuNP on ALT and AST levels in CCl_4-induced HepG2 cells

There was a significant increase in ALT and AST levels with CCl_4 exposure compared to the control group. PEITC-AuNPs and PEITC decrease ALT and AST levels ($p<0.05$). This reduction was greater with PEITC-AuNPs (Figure 2).

Table 1: Particle size distributions of AuNP.

Material	Weighted particle size distributed d, nm ($\bar{x}$ ± SD, n=3)
Au NP	97.57 ± 3.572

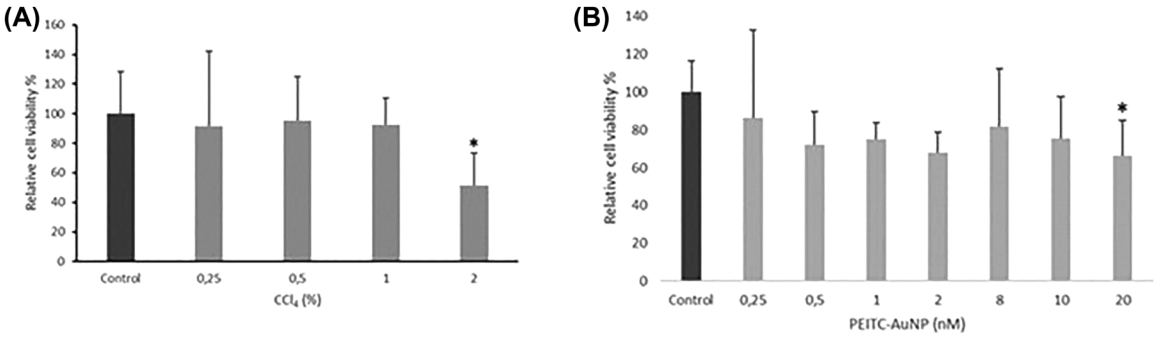

Figure 1: Cytotoxic activities of CCl_4 (A), and PEITC-AuNP administration on HepG2 cells (B). *p<0.05 vs. control.

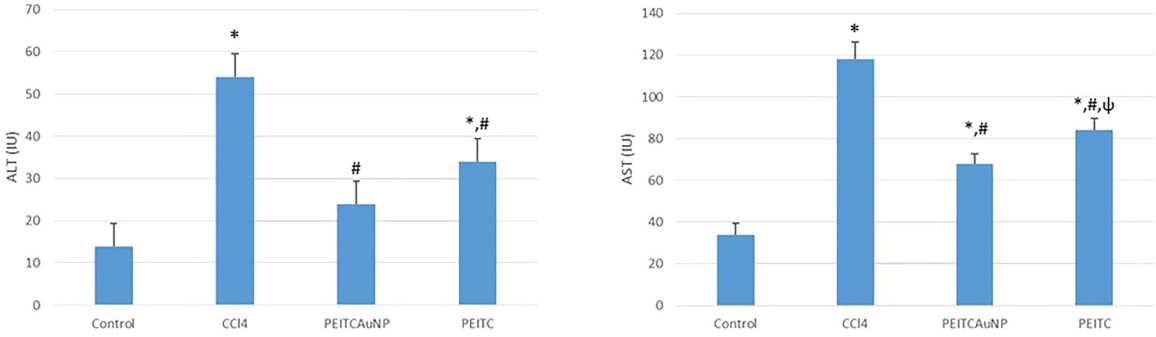

Figure 2: Effect of PEITC-AuNP on ALT and AST levels in CCl4-induced HepG2 cells. Data are presented as mean ± S.E. *p<0.05 vs. control; #p<0.05 vs. CCl_4; ψp<0.05 vs. PEITC-AuNP.

Effect of PEITC-AuNP on Nrf2 level in CCl_4-induced HepG2 cells

As seen Figure 3A, Nrf2 levels decreased statistically significantly in HepG2 cells with CCl_4 administration compared to control (p<0.05). Although the Nrf2 level increased when PEITC-AuNPs was administered, this increase was not statistically significant (p>0.05). PEITC, on the other hand, did not have a positive effect to increase the Nrf2 level.

Effect of PEITC-AuNPs on total antioxidant capacity in CCl_4-induced HepG2 cells

Total antioxidant capacity of HepG2 cells decreased statistically significantly with CCl_4 administration compared to control (p<0.05). Total antioxidant capacity of both PEITC-AuNPs and PEITC application increased statistically significantly compared to CCl_4 group (p<0.05) (Figure 3B).

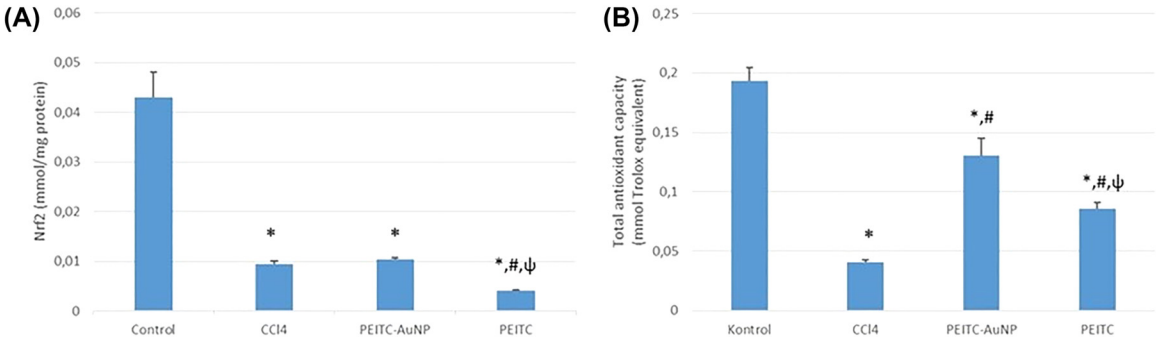

Figure 3: (A, B): Effects of PEITC-AuNP and PEITC on Nrf2 and total antioxidant capacity levels in CCl4-treated HepG2 cells. Data are presented as mean ± S.E. *p<0.05 vs. control; #p<0.05 vs. CCl_4; ψp<0.05 vs. PEITC-AuNP.

Discussion

We observed that the synthesized PEITC-AuNPs presented a better hepatoprotective effect than PEITC through activating Nrf2 in the CCl_4-induced hepatotoxicity in HepG2 cells. Researches have represented that Nrf2 activation protected against hepatotoxic agents leading oxidative injury [19, 20]. It was presented that cyanidin 3-O-glucoside protected against oxidative damage by activating the Nrf2/ARE signalling [21]. Additionally, some natural products such as curcumin, naringenin, and quercetin have been revealed hepatoprotective effect via the activation of Nrf2 signalling [22–24].

It has been reported that PEITC has an chemopreventive, antioxidant, and anticancer effect through activating Nrf2/ARE binding [25]. PEITC is quickly catabolized by glucuronidation due to it is not soluble in water and it is needed at high doses for its chemoprevention effect [26]. These disadvantages caused to research on the improvement of an efficient carrier systems for PEITC, and the acquirement of its curative doses in target cells. Due to their high surface area/volume ratio, the inherent bioinertness and adaptable surface chemistry of AuNPs allow intense drug loading [27]. In addition, the surface modification of AuNPs enables them to be used as a multidirectional drug carrier. According to our cytotoxicity results, a dose-and time-dependent decrease was observed in the viability of Hep-G2 cells exposed to different doses of PEITC-AuNPs. CCl_4 led to cell death significantly and PEITC-AuNPs exposure moderately increased cell viability. We found that AST and ALT levels increased in the CCl_4-administered group. PEITC-AuNPs exposure decreased ALT and AST levels more than PEITC administration. We can say that the PEITC-AuNPs are more effective in reducing liver damage. At the same time, our data also revealed that PEITC-AuNPs presented higher effects on Nrf2 activation and antioxidant capacity compared with PEITC. Although there were studies investigating the biological activities of gold nanoparticles loaded with various natural products such as curcumin, and quercetin that demonstrated more effective than pure compounds, studies with PEITC-AuNPs have been limited for comparison [28].

Some different nano delivery systems have been advanced for PEITC including nanoliposomes, nanocomposites, and nanosheets [29]. It was reported that PEITC nanoliposomes enhanced PEITC capacity to diminish oxidative stress and DNA damage induced by cigarette smoke condensate in bronchial epithelial cells [30]. Additionally, a liposomal nanoparticle with co-encapsulated PEITC and cisplatin presented that more toxic effective against non-small cell lung cancer [31]. The graphene oxide (GO)-PEITC nanocomposite showed a higher anticancer activity compared to the pure PEITC [32]. The black phosphorus nanosheets-based drug delivery system combined with PEITC decreased mutant p53 levels and impeded chemotherapy-resistant tumour cells [33].

A notable treatment approach in many diseases, including cancer, is the usage of biologically active phytochemicals in natural products. Although they offer antioxidant and anticancer effects, they some have disadvantages mainly due to low bioavailability and water solubility reasons. Nanotechnology is appearing as a hopeful drug delivery system model in liver diseases.

Metallic nanoparticles are being actively explored in the delivery of next-generation therapeutics. Many of the drawbacks of conventional therapy, such as side effects and development of resistance, can be overcome with the use of these drug delivery systems. More importantly, it can also enable clinicians to monitor treatment progress and success during treatment. In this context, we plan to conduct further research in these areas in the light of the information obtained from our study.

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References

1. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70:151–71.
2. Marcellin P, Kutala BK. Liver diseases: a major, neglected global public health problem requiring urgent actions and large-scale screening. Liver Int 2018;38:2–6.
3. Hung WL, Yang G, Wang YC, Chiou YS, Tung YC, Yang MJ, et al. Protective effects of theasinensin a against carbon tetrachloride-induced liver injury in mice. Food Funct 2017;8:3276–87.
4. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 2003;33:105–36.
5. Scholten D, Trebicka J, Liedtke C, Weiskirchen R. The carbon tetrachloride model in mice. Lab Anim 2015;49:4–11.
6. Jeong WS, Jun M, Kong AN. Nrf2: a potential molecular target for cancer chemoprevention by natural compounds. Antioxidants Redox Signal 2006;8:99–106.

7. Liu J, Wu KC, Lu YF, Ekuase E, Klaassen CD. Nrf2 protection against liver injury produced by various hepatotoxicants. Oxid Med Cell Longev 2013;2013:305861.
8. Li F, Wang JY. Targeted delivery of drugs for liver fibrosis. Expet Opin Drug Deliv 2009;6:531–41.
9. Leão TK, Ribeiro DL, Machado ART, Costa TR, Sampaio SV, Antunes LMG. Synephrine and caffeine combination promotes cytotoxicity, DNA damage and transcriptional modulation of apoptosis-related genes in human HepG2 cells. Mutat Res Genet Toxicol Environ Mutagen 2021;868–869:503375.
10. Park HJ, Lee SM, Kim HS, Kim JY, Lee SH, Jang JS, et al. Hepatoprotective effect of meal replacement seeds juice based on sweet potato (MRSJ) against CCl4-induced cytotoxicity in HepG2 cells. Food Sci Biotechnol 2019;29:693–704.
11. Dayalan Naidu S, Suzuki T, Yamamoto M, Fahey JW, Dinkova-Kostova AT. Phenethyl isothiocyanate, a dual activator of transcription factors NRF2 and HSF1. Mol Nutr Food Res 2018;62:e1700908.
12. Wang J, Shi K, An N, Li S, Bai M, Wu X, et al. Direct inhibition of GSDMD by PEITC reduces hepatocyte pyroptosis and alleviates acute liver injury in mice. Front Immunol 2022;13:825428.
13. Ma X, Hui H, Jin Y, Dong D, Liang X, Yang X, et al. Enhanced immunotherapy of SM5-1 in hepatocellular carcinoma by conjugating with gold nanoparticles and it's in vivo bioluminescence tomographic evaluation. Biomaterials 2016;87:46–56.
14. Gong R, Chen G. Preparation and application of functionalized nano drug carriers. Saudi Pharmaceut J 2016;24:254–7.
15. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small 2005;1:325–7.
16. Samadian H, Hosseini-Nami S, Kamrava SK, Ghaznavi H, Shakeri-Zadeh A. Folate-conjugated gold nanoparticle as a new nanoplatform for targeted cancer therapy. J Cancer Res Clin Oncol 2016;142:2217–29.
17. Pan A, Zhong M, Wu H, Peng Y, Xia H, Tang Q, et al. Topical application of keratinocyte growth factor conjugated gold nanoparticles accelerate wound healing. Nanomedicine 2018;14:1619–28.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.
19. Luo DD, Chen JF, Liu JJ, Xie JH, Zhang ZB, Gu JY, et al. Tetrahydrocurcumin and octahydrocurcumin, the primary and final hydrogenated metabolites of curcumin, possess superior hepatic-protective effect against acetaminophen-induced liver injury: role of CYP2E1 and Keap1-Nrf2 pathway. Food Chem Toxicol 2019;123:349–62.
20. Lin ZH, Chan YF, Pan MH, Tung YC, Su ZY. Aged citrus peel (chenpi) prevents acetaminophen-induced hepatotoxicity by epigenetically regulating Nrf2 pathway. Am J Chin Med 2019;47:1833–51.
21. Chen L, Li K, Liu Q, Quiles JL, Filosa R, Kamal MA, et al. Protective effects of raspberry on the oxidative damage in HepG2 cells through Keap1/Nrf2-dependent signaling pathway. Food Chem Toxicol 2019;133:110781.
22. Xu D, Hu L, Su C, Xia X, Zhang P, Fu J, et al. Tetrachloro-p-benzoquinone induces hepatic oxidative damage and inflammatory response, but not apoptosis in mouse: the prevention of curcumin. Toxicol Appl Pharmacol 2014;280:305–13.
23. Esmaeili MA, Alilou M. Naringenin attenuates CCl4 -induced hepatic inflammation by the activation of an Nrf2-mediated pathway in rats. Clin Exp Pharmacol Physiol 2014;41:416–22.
24. Sun L, Xu G, Dong Y, Li M, Yang L, Lu W. Quercetin protects against lipopolysaccharide-induced intestinal oxidative stress in broiler chickens through activation of Nrf2 pathway. Molecules 2020;25:1053.
25. Sarkar R, Mukherjee S, Biswas J, Roy M. Phenethyl isothiocyanate, by virtue of its antioxidant activity, inhibits invasiveness and metastatic potential of breast cancer cells: HIF-1α as a putative target. Free Radic Res 2016;50:84–100.
26. Xu C, Li CY, Kong AN. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res 2005;28:249–68.
27. Ghosh P, Han G, De M, Kim CK, Rotello VM. Gold nanoparticles in delivery applications. Adv Drug Deliv Rev 2008;60:1307–15.
28. Vemuri SK, Banala RR, Mukherjee S, Uppula P, Gpv S, AV GR, et al. Novel biosynthesized gold nanoparticles as anti-cancer agents against breast cancer: synthesis, biological evaluation, molecular modelling studies. Mater Sci Eng C Mater Biol Appl 2019;99:417–29.
29. Wang Q, Bao Y. Nanodelivery of natural isothiocyanates as a cancer therapeutic. Free Radic Biol Med 2021;167:125–40.
30. Pulliero A, Wu Y, Fenoglio D, Parodi A, Romani M, Soares CP, et al. Nanoparticles increase the efficacy of cancer chemopreventive agents in cells exposed to cigarette smoke condensate. Carcinogenesis 2015;36:368–77.
31. Sun M, Shi Y, Dang UJ, Pasqua AJD. Phenethyl isothiocyanate and Cisplatin Co-encapsulated in a liposomal nanoparticle for treatment of non-small cell lung cancer. Molecules 2019;24:801.
32. Seema DMJ, Saifullah B, Selvanayagam M, Gothai S, Hussein MZ, Subbiah SK, et al. Designing of the anticancer nanocomposite with sustained release properties by using graphene oxide nanocarrier with phenethyl isothiocyanate as anticancer agent. Pharmaceutics 2018;10:109.
33. Wu F, Zhang M, Chu X, Zhang Q, Su Y, Sun B, et al. Black phosphorus nanosheets-based nanocarriers for enhancing chemotherapy drug sensitiveness via depleting mutant P53 and resistant cancer multimodal therapy. Chem Eng J 2019;370:387–99.

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