THE HEPATOPROTECTIVE ACTIVITY IN MICE OF GOLD NANOPARTICLES/*β*-GLUCAN PREPARED BY GAMMA Co-60 IRRADIATION

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Abstract: Gold nanoparticles (AuNPs) with average particle sizes of 13.3 nm were synthesized by γ-rays irradiation of 1.0 mM Au^{3+} solutions using low molecular weight *β*-glucan extracted from yeast cell wall as a stabilizer. The UV-Vis spectra and TEM images were used to analyze the optical characteristic and particle size of AuNPs. The hepatoprotective activity of AuNPs was tested in acetaminophen induced hepatotoxic mice with an average body weight of ~30 g/head by subcutaneous injection with concentrations of 0.05-0.5 mg/head. The results show that both Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) indexes in blood of tested mice decreased by the increase of injected concentration. Inparticularly, the injection of above concentration decreased ALT index from 49.3 to 78.9% and AST index from 67.4 to 85.5% compared with those of the control without injection. In the APAP induced hepatotoxic group mice injected with 0,25 mg AuNPs, the ALT and AST indexes in blood of tested mice was respectively decreased in 78.9 and 85.0% compared to that in blood of mice induced hepatotoxic and injected with only distilled water. The results show that AuNPs product synthetized by γ-rays irradiation may potentially be developed to apply as a hepatoprotective liver agent.

Keywords: *Acetaminophen, β-glucan, gold nanoparticles, γ-irradiation, hepatoprotective activity*

I. INTRODUCTION

Nowadays, AuNPs have successfully attracted the attention and promised its effects and benefits on biosensor, bioimaging, and biomedical applications such as disease therapeutics, diagnostics, photothermal therapy, targeted delivery and cancer treament [1]. Some studies have showed that AuNPs could absorb light and transfer it to thermal energy, so AuNPs conjugating to cancer cells can be heated by laser pulses to be used as the treatment for tumors [2]. AuNPs have been attached to EGFR antibody (Epidermal growth factor receptor) help antibody add strongly to cancer cells (higher 600 times more than normal cells) and kill cancer cells without affecting normal cells [3]. In addition, AuNPs can be used in some methods to eliminate the tumour in a short time [4]. Moreover, AuNPs can be applied to increase the production of hepatocytes when they are denatured in association with cysteamine in culture medium liver cells [5].

Gamma ray irradiation is considered as an effective method due to several advantages such as: (1) the reaction can be carried out at room temperature; (2) yield of AuNPs is high; (3) AuNPs is purely prepared without contamination of excessive chemical reductant and Au^{3+} ions residue; (4) the size of AuNPs is easily controlled by varying Au^{3+} ions or seed enlargement approach [6, 7]; (5) mass production can be carried out and (6) processing is satisfied to requirement of clean production [8].

In the present study AuNPs/*β*-glucan prepared by gamma ray (Co-60) irradiation was carried out and investigated its hepatoprotective activity in Swiss mice aiming to apply as a special hepatoprotective material.

II. EXPERIMENTAL

II.1. Materials and methods

Materials

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4.3H_2O$) was purchased from Merck, Germany. Low molecular weight *β*-glucan extracted from yeast cell wall with Mw ~ 25 kDa was prepared at Bio-material and Nano Technology Department, Biotechnology Center of Ho Chi Minh City, Viet Nam. Acetaminophen (APAP) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Methods

Synthesis of AuNPs/β-glucan by gamma ray irradiation

The colloidal AuNPs with a concentration of 1.0 mM stabilized in 0.5% *β*-glucan were prepared as follow: the stock solution of 10 mM Au^{3+} was added to 2% *β*-glucan at an appropriate ratio with distilled water, and then adjusted pH ~ 8 by NH_4OH 2,5%. Subsequently, the above solutions were irradiated at 6 kGy by a gamma Co-60 GC-5000 sources (BRIT, India) at Biotechnology Center of Ho Chi Minh City with a dose rate of 10 kGy/h.

Characterization of AuNPs/β-glucan

The optical characteristics and particle size of AuNPs were determined by UV-Vis spectrometry method and Transmission Electron Microscope (TEM) images, respectively [7]. The UV-Vis spectra of 0,1 mM AuNPs samples measured by a UV-Vis spectrophotometer model Genesys 10S UV-Vis (Thermo, USA). The AuNPs size and morphological characteristics were identified by a JEM 1400, JEOL (Japan) and statistically calculated from about 300 particles followed the method described by Aryal et al [9].

Determination of hepatoprotective effects of AuNPs in APAP induced hepatotoxic mice

Mice were supplied by Pasture Institute in Ho Chi Minh City and fed for 8 weeks and their average body weight was about 30 g.

In total, 144 female mice were randomly designed into two groups of 72 mice each: Normal group and APAP induced hepatotoxic group. Each group underwent eight experiments consisting of 9 mice. Each mouse was tail vein injected with 0.2 mL AuNPs/*β*-glucan solution containing from 0 (only irradiated *β*-glucan) to 0,5 mg/head. All animals were injected three times after every 7 days. The control experiment received only water injection. On 22nd day, mice in the "APAP induced hepatotoxic group" were injected with APAP at a dose of 500 mg/kg body weight followed by Morsy et al. [10]. The mice after intravenous injection with APAP 24 h, 0.5 mL blood from the myocardium of tested mice was collected and put into tubes containing heparin for analysis. The collected blood was then centrifuged at 4500×g for 10 min to separate the serum. The obtained serum was then used for analyzing the serum's clinical chemistry indexes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by a Beckman Coulter Au480 analyzer (USA).

All experiments were carried out in triplicate and data were statistically analyzed using the analysis of variance (ANOVA) test.

II.2. Results

Synthesis and characterization off AuNPs/*β*-glucan

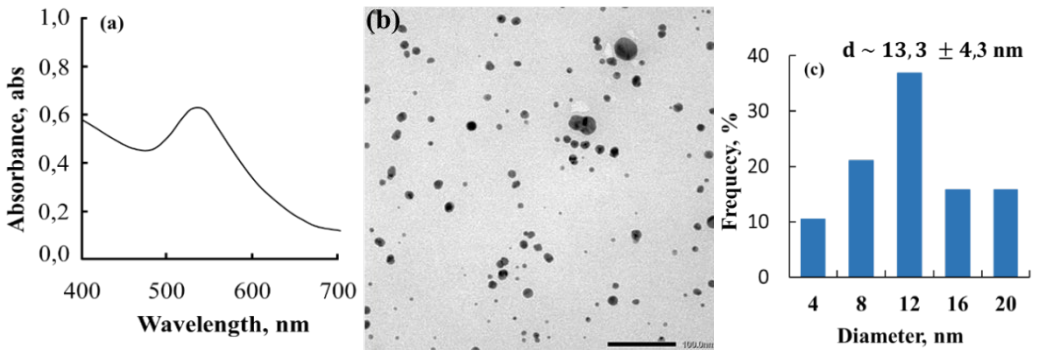

Figure 1. UV-vis spectra (a), TEM image (b) and histogram of particle size distribution (b) of AuNPs prepared by γ-rays irradiation method

Effect of AuNPs/β-glucan on AST index in mice

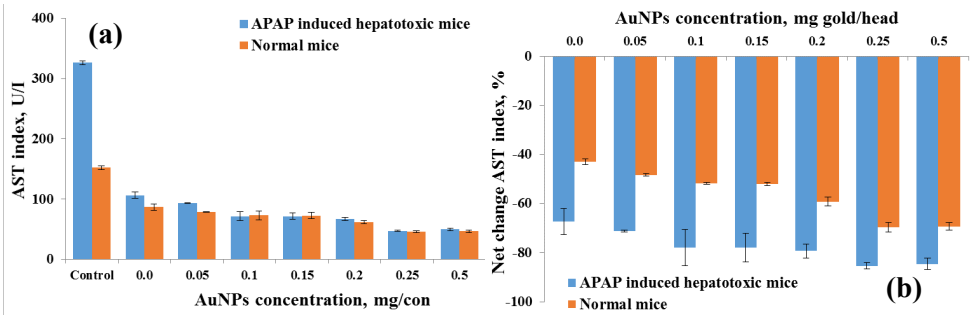

Figure 2. The reduction of AST index in the blood of APAP induced hepatotoxic and normal mice (a) and net change AST index (b) by subcutaneous injection of AuNPs at various doses

Effect of AuNPs/*β*-glucan on ALT index in mice

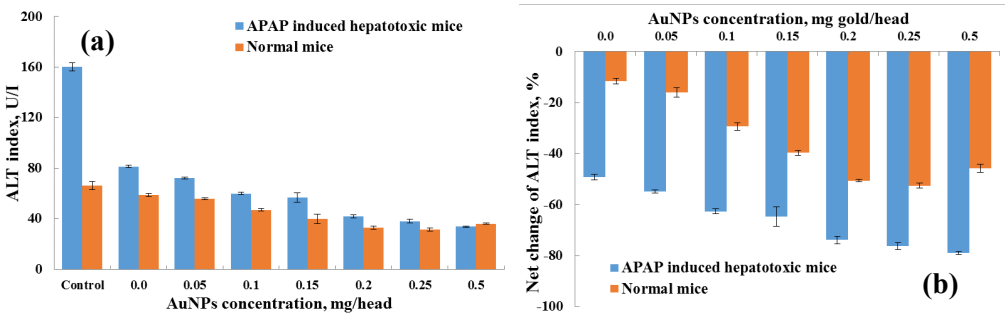

Figure 3. The reduction of ALT index in the blood of APAP induced hepatotoxic and normal mice and net change ALT index by subcutaneous injection of AuNPs at various doses

II.3. Results and Discussion

Synthesis and characterization off AuNPs/*β*-glucan

The UV-Vis spectrum of AuNPs solution in Figure 1a showed the maximum absorption wavelength (λ_{max}) at 521 nm and this peak is the characteristic of the surface plasmon resonance band of AuNPs and preliminary confirmed the formation of gold nanoparticles [7,11]. The TEM image and particles size distribution are shown in figures 1b and 1c. The average diameter of AuNPs was determined to be about 13.3 nm with a fairly narrow distribution.

Effects of AuNPs/*β*-glucan on AST index in mice

The hepatoprotective activity of AuNPs was tested in Swiss mice by subcutaneous injection. The results in Figure 2 show that after injecting AuNPs/*β*-glucan with concentrations of 0 - 0.5 mg/head, the AST index of mice in both normal and APAP induced hepatotoxic groups of mice were decreased. Particularly, AST index in the APAP induced

hepatotoxic group was found from 326.1 to 47.4 U/L and from 152.1 to 46.3 U/L in normal mice.

In the APAP induced hepatotoxic group (Figure 2a), the injection of 0.25 mg/head AuNPs strongly reduced the AST index to 85.5% and 67.4% compared to those in the control mice (injected with only water) and in the mice injected with irradiated *β*-glucan (without AuNPs), respectively. These results indicated that AuNPs had strong effect on the decreasing AST index in APAP induced hepatotoxic mice and the dose of 0.25 mg/head was the optimum effective for reduction of AST in in mice.

On the other hand, the colloidal AuNPs/*β*-glucan product also showed an effect on lowering the AST index in the blood of normal mice. The injection of AuNPs with doses of 0.25 - 0.5 mg/head reduced about 69% the AST index compared to that in the control mice (Figure 2b).

Effects of AuNPs/*β*-glucan on ALT index in mice

Besides AST, ALT is also a large quantities enzyme founded in the liver and this enzyme plays an important role in metabolism. ALT is usually increased in blood when liver cells were damaged. In this study, there was a big difference on ALT index between normal and APAP induced hepatotoxic mice.

The results in Figure 3a showed that in normal group, the ALT index was in range of 31.5 to 66.4 U/L and the lowest (31.5 U/L) of this value was found in blood injected with 0.25 mg/head only.

In the hepatotoxicity group, the results from Figure 3a show that the ALT index was decreased by the increase of AuNPs concentration. In particularly, the ALT index in blood of mice injected with 0 - 0.5 mg/head were found at 81.3 - 33.7 U/L, respectively. Compared to the control, the injection of 0.25 mg/head was reduced to 78.9% of the AST value in blood of tested mice (Figure 3b).

It can be seen from the results of this study that the tail vein injection with 0.25 mg/head of AuNPs/*β*-glucan preparations by gamma Co-60 strongly reduced the AST and ALT index in APAP induced hepatotoxic mice. Negahdary, et al. has also demonstrated that gold nanoparticles with a particle size of about 10 nm also had the effect of reducing the CAT and GPX index in Wistar mice [12].

CONCLUSION

AuNPs with particle sizes about 13.3 nm was successfully synthesized by γ-irradiation using *β*-glucan extracted from the yeast cell wall as the stabilizer. AuNPs/*β*-glucan displayed a strongly reduction of ALT and AST indexes in APAP hepatotoxic mice after 21 days injecting with 0.25 mg/head. The results show that AuNPs product synthetized by gamma-irradiation may potentially be developed to apply as a hepatoprotective liver agent.

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HOẠT TÍNH BẢO VỆ GAN Ở CHUỘT NHẮT CỦA CHẾ PHẨM VÀNG NANO/β-GLUCAN CHẾ TẠO BẰNG PHƯƠNG PHÁP CHIẾU XẠ GAMMA Co-60

Tóm tắt: Vàng nano (AuNPs) có kích thước hạt khoảng 13,3 nm được chế tạo bằng phương pháp chiếu xạ tia γ dung dịch 1,0 mM Au^{3+} sử dụng β-glucan nấm men có khối lượng phân tử thấp làm chất ổn định. Phương pháp đo phổ UV-Vis và chụp ảnh TEM được sử dụng để xác định các đặc trưng của các hạt AuNPs. Hoạt tính bảo vệ gan của chế phẩm AuNPs được khảo sát trên chuột nhắt đã gây độc gan bằng acetaminophen có trọng lượng trung bình ~ 30 g/con với liều tiêm dưới da là 0,05 đến 0,5 mg/con. Kết quả phân tích cho thấy cả 2 chỉ số Alanine-aminotransferase (ALT) và Aspartate-aminotransferase (AST) trong máu chuột đều có xu hướng giảm khi tăng liều tiêm của AuNPs dần. Cụ thể là với liều lượng tiêm như trên đã làm giảm tương ứng chỉ số ALT từ 49,3 đến 78,9% và AST từ 67,4 đến 85,5% so với đối chứng không tiêm AuNPs. Ở nhóm chuột gây độc gan bằng APAP, khi tiêm AuNPs vời liều 0,25 mg/con, chỉ số AST và ALT trong máu đã giảm tương ứng là 78,9 và 85,0% so với chuột gây độc gan đối chứng chỉ tiêm nước cất. Kết quả của nghiên cứu này cho thấy chế phẩm AuNP chế tạo bằng phương pháp chiếu xạ tia γ có tiềm năng ứng dụng làm chất bảo vệ gan.

Từ khoá: *Acetaminophen, bảo vệ gan, vàng nano, chiếu xạ gamma, β-glucan*