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Research Article

Investigation into the Anti-oxidant and Anti-Tumor Activity of the Bovine Serum Albumin Nanoparticles Containing Grape Seed Polyphenols Fabricated by Desolvation Method

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Abstract

Grape seed polyphenols (GPP) have been known to have various pharmaceutical activities including anti-oxidant and anti-tumor activity. However, its clinical application is hindered by low stability and bioavailability. One of the most common methods for solving these problems is to use different types of carriers. Recently, protein based drug delivery system has gained a great interest. In this study, bovine serum albumin (BSA) nanoparticles loaded GPP were manufactured by desolvation method. Several characteristics such as the average size, shape, releasing behavior and stability under UV light were tested by different techniques. Anti-oxidant activity of BSA-GPP nanoparticles were compared to free GPP. In addition, anti-tumor activity was also studied in mice model injected sarcoma-180 cells. The result showed that the shape of prepared BSA-GPP nanoparticles was spherical with the average size of 207.02nm. GPP were released by a diffusion from the nanoparticles. The nanoparticles loaded GPP showed relatively higher stability under UV condition. BSA-GPP nanoparticles maintained longer in vitro antioxidant activity than free GPP. Importantly, tumor inhibition rate of BSA-GPP nanoparticles was 84%, which was much higher than that of free GPP. In summary, BSA nanoparticles had a high potential to be used as a carrier for improving stability and increasing bioavailability of GPP.

Keywords

Grape Seed Polyphenols, Bovine Serum Albumin, Nanoparticles, Bioavailability, Stability

1. Introduction

Grape seed polyphenols (GPP) are known to have various pharmaceutical activities such as anti-oxidant, anti-tumor, anti-diabetes, anti-bacteria, ant-aging and cardiovascular protection [1-3]. The strong anti-oxidant activity of grape seed polyphenols has been reported in prior studies [2, 3]. Generally, phenolic compounds could neutralize free radicals by providing electrons or hydrogen to reactive oxygen species, thereby they had anti-oxidant activity. In addition, aromatic rings in phenolic compounds could localize free electrons. Grape seed polyphenols also exhibit anti-tumor activities through several mechanisms such as regulation of oxidative stress, induction of apoptosis and interaction with

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inflammatory and cancer metabolic pathways. They showed inhibitory activities against many cancer cells including lung cancer cells, skin cancer cell lines and so on [4-6].

Despite its pharmaceutical values, clinic application of GPP has some major drawbacks: mainly low stability and bioavailability [1, 7]. Polyphenols are sensitive to environmental changes. They readily become oxidized by reacting with dissolved oxygen in water or oxygen in air due to the existence of hydroxyl groups. Bioavailability of polyphenols is also very low. Generally, adults take in 1 gram of polyphenols per day through their diet but its concentration in plasma is usually below 1 μM. The main causes of low bioavailability could be poor water solubility, instability in human gut, rapid metabolite rate and so on [1, 2, 7, 8].

These problems can be solved by using various kinds of carriers. The application of carriers could enhance biocompability and prevent the degradation of bioactive substances or their binding to other substances inside the body. In addition, it also could control the release of bioactive materials in target tissues or cells [9-11].

In recent years, protein-based carrier system has been attained great interests of many scientists because of their advantages of enhancing solubility and stability, loading both hydrophilic and hydrophobic bioactive materials and easy modification. Successful examples of carrier materials are albumin, casein, collagen, gelatin, zein, fibrin, elastin, soy protein, whey protein etc [12].

Among them, albumin is one of the most widely used carrier materials due to a number of attractive features such as high solubility and biocompability, long half-life, many binding sites for both hydrophilic and hydrophobic substances and easy modification [13-16]. In previous literature, various approaches have been introduced to increase stability and bioavailability of polyphenols by using albumin. For example, in previous study [17], the author prepared nanoparticles by self-assembly method using bovine serum albumin and green tea polyphenols. These particles were mainly localized MCF-7 cells. In another study, dispersibility, stability and anti-oxidant activity of resveratrol were improved by using BSA [18].

However, in reviewing the literature, no data was found on the improvement of GPP by using BSA. Therefore, the present study set out with the purpose of preparing BSA nanoparticles containing the GPP and assessing its anti-oxidant and anti-tumor activity.

2. Materials and Methods

2.1. Isolation of Grape Seed Polyphenols (GPP)

GPP were isolated according to previous report with slight modifications [19]. Briefly, 100 g of dried grape seed obtained from a winery factory were milled and extracted with 1 liter of 50% ethanol for 18h. Then, this solution was filtered through filter paper. The residue was further extracted with 300ml 95% ethanol for 12h and filtered again. The final solution was joined and concentrated in a rotary evaporator. The polyphenol content was measured by the Folin-Ciocalteo method using gallic acid as standard.

2.2. Manufacture of BSA Nanoparticles Loaded GPP

BSA nanoparticles were prepared by desolvation method according to the previous method with some modifications [20]. First, 4mg of BSA was dissolved in 25 mL of double distilled water. On the other hand, 10mg of GPP was dissolved in 95% ethanol. Then, GPP solution was added to BSA solution drop by drop under constant magnetic stirring (300 rpm) until the solution became turbid. Then, 1 ml of 0.5% glutaraldehyde was added. After continuous stirring for 1 hour, this solution was centrifuged at 1 2000 rpm for 20 min. The GPP concentration in supernatant was measured by the Folin-Ciocalteo method in order to determine encapsulation efficiency calculated by the following equation [21].

Encapsulation Efficiency (%) = [GPP (total)- GPP (in supernantant)]/ GPP (total) ×100

Precipitant was resuspended in 20 mL of double distilled water.

2.3. Characterization of BSA Nanoparticles Loaded GPP

2.3.1. Size Distribution and Morphology

The average size of the nanoparticles was measured by a laser scattering particle size analyzer (BT-90). The morphology of BSA containing the GPP was observed by atomic force microscopy (CSPM-5500).

2.3.2. In vitro Release Characteristics

GPP and BSA-GPP nanoparticles corresponding the same amount of GPP were dissolved in Tris buffer (pH 7.4) and placed in the dialysis membrane with sealing the both ends. This dialysis membrane was placed in a beaker containing 50 mL of Tris buffer (pH 7.4). Then, 3 ml of the solution was taken every 2 hours in order to measure GPP content under the constant magnetic stirring (160 rpm) at 37℃. Some releasing models were used to simulate its releasing behavior [22, 23].

2.3.3. Stability Under UV Light

The stability of the BSA-GPP nanoparticles under UV light was studied by reference to previous study [21]. A certain amount of GPP and BSA-GPP nanoparticles equivalent to GPP were diluted with 10 mL of distilled water and placed under the UV lamp (254 nm) in the distance of 30 cm. Then, an aliquot (0.5 mL) was taken every 2 hours to measure GPP content by Folin-Ciocalteo method.

2.4. Anti-oxidant and Anti-Tumor Activity Test

First of all, various concentration of GPP samples and BSA-GPP nanoparticles equivalent to GPP samples were prepared. BSA solution without GPP was used as a blank.

2.4.1. DPPH Assay

DPPH free radical scavenging activity of GPP and BSA-GPP nanoparticles were compared according to previous study [24]. Briefly, GPP solutions with different concentrations were placed in test tubes and 3 mL of DPPH was added in each tube. On the other hand, 1 mL of BSA-GPP nanoparticles equivalent to GPP concentration prepared above was sonicated for 10 min, and put in test tubes, followed by 3 mL of DPPH. BSA solution without GPP was used as a blank. After 30 min incubation in dark, the absorbance was measured at 517 nm using a UV-Vis spectrometer. The radical scavenging rate was calculated by following equation.

$I(\%) = [(A_o-(A-A'))/A_o] \times 100$

I: DPPH Radical Scavenging Rate

A_o: Absorbance in Control (Distilled water instead of sample)

A': Absorbance in Blank (Distilled water instead of DPPH)

A: Absorbance in Test

2.4.2. Superoxide Anion Radical Method

Superoxide anion scavenging ability was measured using pyrogallol [24]. 0.1 mL of 6 mM pyrogallol was added to various concentration of GPP and BSA-GPP nanoparticles after 10 min sonication. Absorbance was measured at 325 nm after 5 min.

2.4.3. Tumor Inhibition Assay

Twenty-four mice injected sarcoma-180 tumor cells were used in order to test the anti-tumor activity. The mice were divided into three groups: GPP group (fed only GPP: dose: 200 mg/kg/d), BSA-GPP group (fed nanoparticles: dose: 200 mg/kg/d) and Control group (fed only feed). At day 30, tumor cells were removed and weighted. Tumor suppression rate was according to previous report [25].

2.5. Statistical Analysis

Student's t test and Tukey's HSD Test with a confidence level of 95% were applied. All data were expressed as mean ± SD (standard deviation) and processed with R for windows version.

3. Result and Discussion

3.1. Encapsulation Efficiency, Size Distribution and Morphology

Encapsulation efficiency tested by the equation 1 was 36.8% in this experiment. Figure 1 depicts particle size distribution of BSA-GPP nanoparticles measured by laser particle size analyzer. As shown in the future, more than 50% of the particles had the nano-range size less than 160 nm and the average size was 207.02 nm. In the previous study [20], membrane permeability was increased and particle localization around the tumor were observed when the size of albumin nanoparticles was small as 200 nm. Therefore, it is suggested that the prepared BSA-GPP nanoparticles could be considered to have a high potential for the localization around the tumor cell.

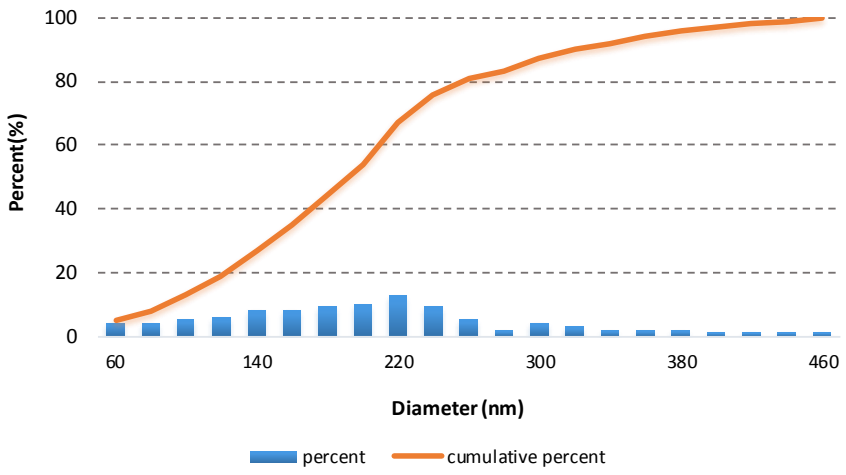

Figure 1. *Size distribution of BSA-GPP nanoparticles.*

The surface morphology and shape of particles play an important role in the transport, absorption and release of

active materials. AFM observation showed that the surface of fabricated BSA-GPP particles was smooth and shape was spherical. There results were similar to previous studies [14, 17].

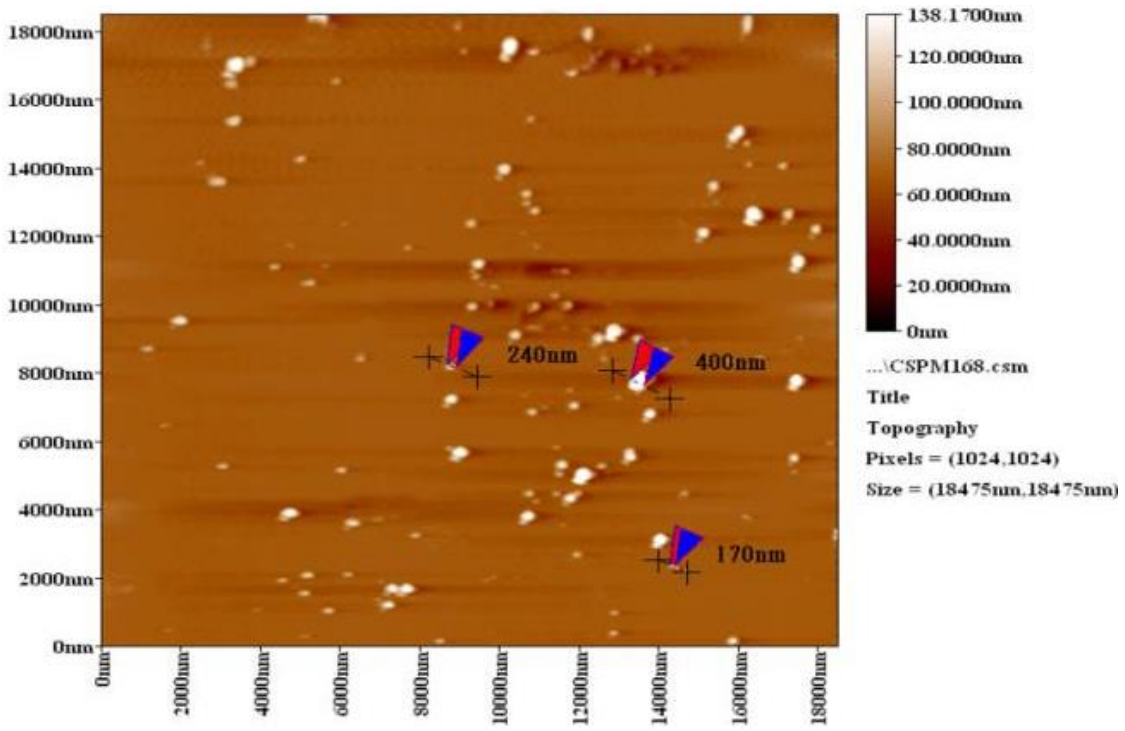

Figure 2. *AFM picture of BSA-GPP nanoparticles.*

3.2. Releasing Behavior of GPP from the Nanoparticles

Releasing behavior of GPP from the nanoparticles was shown in Figure 3. As shown in the figure, the releasing rate increased as time went by. In order to characterize the release kinetics of GPP, several models were used (Table 1). As can be seen, it was supposed that GPP was released according to the Korsmeyer-Peppas model ($Ct=Kt^n$) as the R^2 was the highest. According to the previous report [17], active material was diffused according to Fick's law, if $n<0.43$ when the nanoparticle was spherical. The calculated n value in this experiment was 0.42, suggesting that GPP was released by diffusion according to Fick's law.

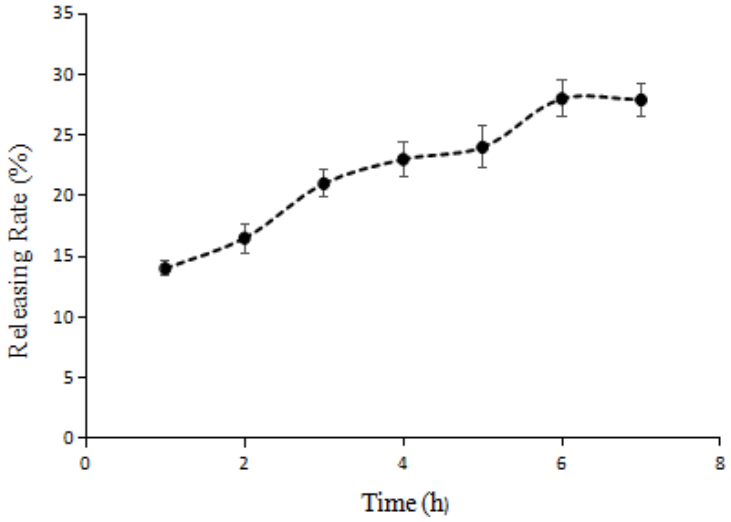

Figure 3. *Releasing Behavior of GPP from the nanoparticles.*

Table 1. *Releasing Models.*

No	Releasing Models	R^2
1	$C_t=C_0-k_0t$ (zero-order)	0.9468
2	$C_t=C_0e^{-k1t}$ (first-order)	0.9053
3	$C_t=K_Ht^{1/2}$ (Higuchi)	0.9705
4	$C_0^{1/3}-C_t^{1/3}=K_{HC}t$ (Hixson-Crowell)	0.9214
5	$C_t=kt^n$ (Korsmeyer-Peppas)	0.9737

3.3. Stability under UV Light

Figure 4 shows the GPP stability under UV light. As can be seen, GPP content decreased over time. However, GPP in nanoparticles was much higher than that of free GPP, especially after 10 hours, with the values of 92.04% and 85.79%, respectively. This indicates the BSA could be used as a carrier to protect GPP from the UV light.

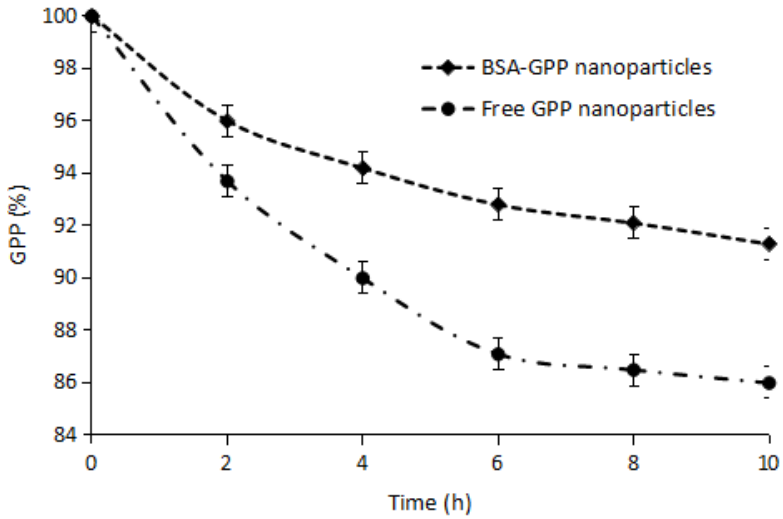

Figure 4. *UV stability of BSA-GPP nanoparticles and GPP.*

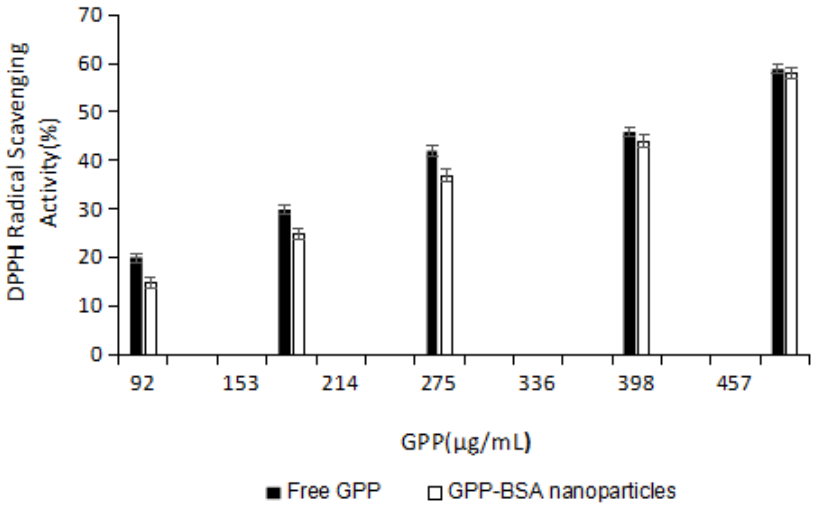

Figure 5. *DPPH radical scavenging activity.*

3.4. DPPH Assay

As shown in figure, free radical scavenging rate increased with the increase of the concentration. Free radical scavenging rate of BSA-GPP nanoparticles at 400 and 500 μg/mL was 96% and 97% of GPP, respectively. From the results, it can be seen that BSA does not inhibit the anti-oxidant activity of GPP.

3.5. Superoxide Anion Scavenging Activity

The results of comparing the superoxide anion scavenging activity of GPP and BSA-GPP nanoparticles are shown in Table 2. The IC 50 values of GPP and BSA-GPP nanoparticles were 353.40 and 358.38μg/mL, respectively. After 3 days, the values were 475.25 and 369.50 μg/mL, respectively, with the NPs having a 1.3-fold stronger activity for the superoxide anion scavenging activity than the free GPP.

Table 2. *Superoxide anion scavenging activity of GPP and BSA-GPP nanoparticles.*

Concentration (μg/mL)	GPP	BSA-GPP	GPP (3d later)	BSA-GPP (3d later)
100	21.18±4.47	20.21±3.13	15.46±3.53	19.18±3.12
200	35.47±6.32	36.06±5.79	25.37±4.38	35.43±2.53
300	44.48±5.68	45.78±8.93	30.63±4.38	46.83±3.57
400	54.74±3.48	53.72±1.53	44.72±3.17	53.35±4.35
500	65.38±2.78	63.69±5.72	52.30±4.11	60.36±4.79
IC_{50}	353.40μg/mL	358.38μg/mL	475.25μg/mL	369.50μg/mL

3.6. Anti-tumor Activity in Mice

Tumor mass and inhibition rates measured after 30 days in the control group, the grape seed polyphenol-fed group and the nanoparticle-fed group are shown in Table 3. As shown in Table 3, the tumor-suppressive rate of the test group fed grape polyphenols was 58.9%, and that of the test group fed nanoparticles was 80.7%, showing a significant tumor-suppressive activity over the free grape polyphenols.

Table 3. *Anti-tumor activity of GPP and BSA-GPP.*

Group	Tumor mass (g)	Inhibition rate (%)
Control	3.35±0.46	-
GPP	1.37±0.21	58.9
BSA-GPP	0.64±0.11	80.7

4. Conclusion

In present study, BSA-GPP nanoparticles were successfully manufactured by desolvation method. The prepared nanoparticles had a spherical shape with the average size of 207.02 nm. GPP was released by a diffusion. BSA-GPP nanoparticles showed a better antioxidant activity such as DDPH radical scavenging activity and super anion scavenging activity. Especially, it exhibited higher tumor inhibition activity than the same dose of GPP in tumor mice model injected with sarcoma-180 cells. This essay highlights the possibility of using BSA for the stability improvement and bioavailability increase of GPP. Further research is need to investigate the exact mechanism of anti-tumor activity.

Abbreviations

GPP	Grape Seed Polyphenols
BSA	Bovine Serum Albumin
DPPH	2,2-diphenyl-1-picrylhydrazyl
AFM	Atomic Force Microscopy

Author Contributions

Su-Chol Rim: Conceptualization, Writing – original draft.
Su-Ung Jang: Methodology, Validation
Gyong-Man Rim: Methodology, Validation
Chol An: Methodology, Validation
Kwang-Il To: Supervision, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interests.

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