medical sciences forum

MDPI

Proceedings

PLGA nanoparticles loaded with cinnamon extract and coated with PVA/poloxamer188 †

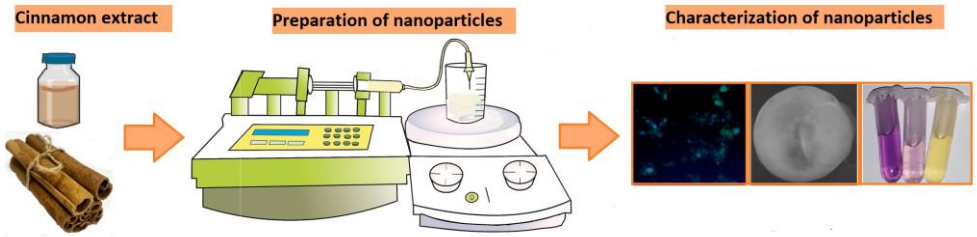
Fateme Madani [1†], Masood Khosravani [1,*] and Mahdi Adabi [1,*]

[1] Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran; f-madani@razi.tums.ac.ir, madabi@tums.ac.ir, drkhosravani@tums.ac.ir

* Correspondence: drkhosravani@tums.ac.ir.

Abstract: Polymeric nanoparticles hold promise as therapeutic drug delivery vehicles. Cinnamon extract has received a lot of attention due to its significant properties such as antibacterial, antifungal, antioxidant, and even anti-cancer properties. The purpose of this study was to create cinnamon extract-loaded PLGA nanoparticles and evaluate their physiochemical characteristics and cytotoxicity against the C6 cell line. Physiochemical characteristics such as mean diameter, zeta potential, drug loading were measured. Antioxidant activity and also cytotoxicity of nanoparticles were investigated by DPPH and MTT studies, respectively. The mean diameter of nanoparticles was 120±24 nm. The antioxidant activity of the cinnamon extract was mostly preserved in nanoparticles and the toxicity effect on cancer cells was investigated.

Keywords: PLGA nanoparticle; cinnamon extract; poloxamer188; PVA



.

Citation: Madani, F.; Khosravani, M.; Adabi, M. PLGA nanoparticles loaded with cinnamon extract and coated with PVA/poloxamer188. **2023**, *3*, x. https://doi.org/10.3390/xxxxx

Published: 17 March 2023

1. Introduction

One of the challenges in treating tumors is the untargeted delivery of chemotherapeutics and their unwanted toxicity to healthy organs. Toxicity from chemotherapy drugs is hazardous and may even lead to tissue damage [1]. Today, with the use of nanotechnology, the targeted delivery of drugs has improved compared to the past [2].

Cinnamon (Cin) is a well-known spice that is also utilized in herbal medicine [3]. In addition of being antioxidant [4], antifungal [5], antibiotic agent [4], it is effective in treating diseases such as obesity [6], Parkinson's [7], cancer, and cardiovascular disease [8]. The effectiveness of this substance on various cancer cells such as leukemia [9], prostate [10], and breast cancer [11] were evaluated.

Polymeric nanoparticles (NPs) are proper nanovehicles for drug delivery, and they can be categorized as either natural or synthetic [12]. Poly(lactic-co-glycolic acid) (PLGA) is an extensively studied and widely used synthetic polymer [13], which has gained prominence due to its biodegradability, biocompatibility, low cost, and FDA approval [14]. In this study we have prepared Cin loaded in PLGA NPs (Cin/PLGA NPs) to study the physiochemical properties and cellular toxicity of NPs.

2. Materials and Methods

PLGA (MW 30.000 g.mol^{-1}, 50:50) was bought from Xi'An Xinlu Biotech company, PVA (Poly(vinyl alcohol)) (MW 30.000 g.mol^{-1}) and poloxamer188 were obtained from Merck. Cinnamon extract was purchased from Adonisherb company. Acetonitrile and PBS were purchased from Dr. Mojallali company. Trypsin, FBS and MTT powder were bought from Sig-ma-Aldrich.

2.1. Preparation of nanoparticles:

Cin/PLGA NPs were prepared by emulsion solvent evaporation method. First of all, 30 mg of PLGA was dissolved in 3 ml acetonitrile and stirred for 10 min at 500 rpm. Then 2 mg of cinnamon extract was added to PLGA solution and this solution was added to 30 ml of 1% PVA/Poloxamer188 (10:1) solution, simultaneously. The emulsion was sonicated for 8 min at 120 W. then it was put on a stirrer for 3 h at 300 rpm. Afterward, it was centrifuged at 10,000 rpm for 25 min and washed two times.

2.2. characterization of nanoparticles

2.2.1. Size distribution and zeta potential

DLS (ScatterScope1) and scanning electron microscope (SEM) were used to check nanoparticles size distribution and zetasizer (Malvern) was also applied for the measurement of nanoparticles zeta potential.

2.2.2. Drug loading (DL) % and Encapsulation Efficiency (EE) %

5. mg of lyophilized sample was solved in 5 ml of acetonitrile and was putted in bath sonication for 5 min. Then the absorption was read at 286 nm. DD% and EE% were calculated.

2.2.3. In vitro drug release

10. mg of lyophilized Cin/PLGA NPs was dispersed in 5 ml of PBS (pH 7.4), and then poured in a dialysis tube. Then was soated in 45 ml of PBS and incubated for 7 days at 100 rpm, 37 °C. At each time point (1, 3, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h) 5 ml of the medium was extracted and fresh medium was replaced.

2.2.4. FTIR

Fourier transform infrared spectroscopy was used to confirm drug encapsulation in nanoparticles from 400-4000 cm^{-1}. PVA, poloxamer188, PLGA, Cin, and Cin/PLGA NPs were used and dispersed with KBr for the pellet preparation.

2.3. Antioxidant activity

DPPH assay was performed to check the antioxidant activity of Cin/PLGA NPs. Serial concentrations of Cin/PLGA NPs, PLGA NPs, and Cin (1 μg/mL to 2000 μg/mL) were treated with DPPH solution in ethanol (2mg/100mL) for 3 h in darkness. Then the absorption of each sample was read at 517 nm by UV-Vis spectroscopy. The percent of antioxidant activity of samples was calculated as bellows:

% Inhibition = ((A0-A1)/A1) x 100 (A0) control, (A1) sample

2.4. Blood compatibility

To check blood compatibility hemolysis assay was applied. Diluted blood was encountered with PLGA NPs and Cin/PLGA NPs for 3 h and absorption was read at 540 nm.

2.5. Cellular uptake of the nanoparticles

Cells were cultured with DMEM-F12 medium containing 10% *v*/*v* and 1% *v*/*v* FBS and penicillin/streptomycin, respectively. 1 mg of Cin/PLGA NPs which were loaded with

carbon quantum dot as a fluorescent agent was dispersed in a sterile PBS and poured on seeded cells. After 3 h, cells were washed with PBS and 4% paraformaldehyde was added, followed by DAPI staining. A fluorescent microscope (Olympus BX43) was applied to obtain photos.

2.6. Cytotoxicity of nanoparticles

C6 Cells were seeded in 96 well plates. Cin and Cin/PLGA NPs were dispersed in PBS solution and added to each well. After 24 and 72 h of the treatment, wells were washed three times with PBS and MTT solution (0.5 mg/mL) was added. After after 3 h DMSO was used and absorptions were read via a microplate reader (Bio Tek).

3. Results

3.1. Physiochemical characterization of Cin/PLGA NPs

Emulsion solvent evaporation method was applied to prepare Cin/PLGA NPs. Cin/PLGA NPs mean diameter was 120±24 (Fig. 1A, B, C). Zeta potential of PLGA NPs and Cin/PLGA NPs were -10.1±1.1 mV and -3.66±1.8 mV (Fig. 1D). EE% and DL% of Cin in Cin/PLGA NPs were calculated using UV/Visible spectroscopy at 286 nm (Fig. 1G), which were 51±5 % and 4.2±0.7 %, respectively. In vitro release of cinnamon from Cin/PLGA NPs demonstrate two phases of drug release. At first, a burst release was seen at first 12 h which was induced to a 16 % release of Cin (Fig. 1E). 23.2%, 31.6% and 44.6% of Cin was released during the first 24, 48 and 72 h. it was observed that the release of Cin was extended to more than 7 days, and 84% of Cin was released after 168 min (7 days).

In PLGA three sharp peaks were seen in 2940 cm^{-1}, 1143 cm^{-1}, and 668 cm-1. In PVA the sharpest peak was at 1065 cm^{-1}. In poloxamer188 the main peaks were at 2882 cm^{-1}, 1099 cm^{-1}, and 1144 cm^{-1}. In the cinnamon extract, the sharpest peak was at 1047 cm^{-1} and two wide bands were observed at 2922 cm^{-1} and 3334 cm^{-1}. In Cin/PLGA NPs the existence of Cin, PLGA, PVA, and poloxamer188 was confirmed according to the various peaks which were obtained (Fig. 1F).

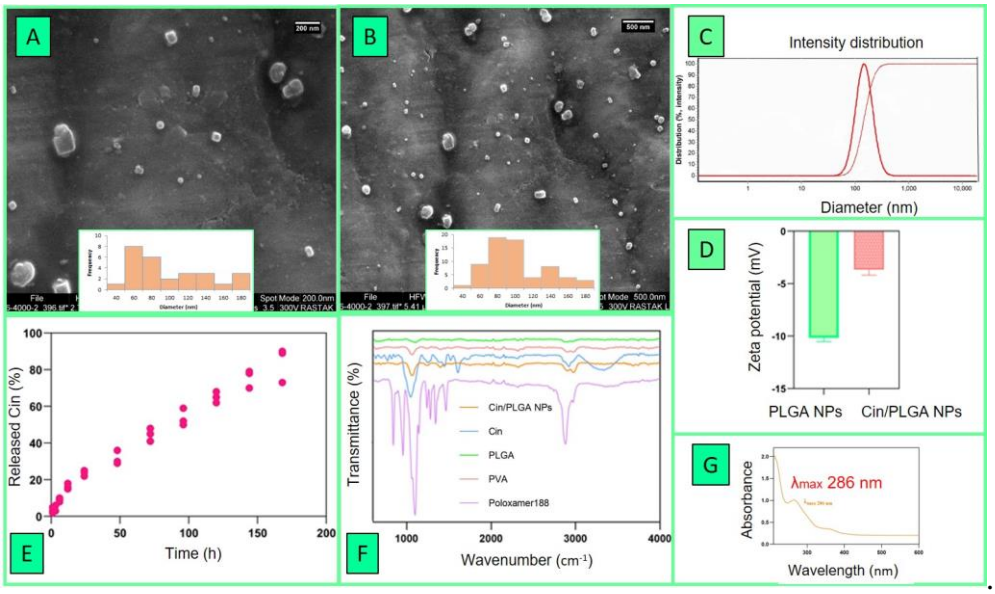


Figure 1. A,B) SEM image of nanoparticles, C)DLS result, D)zeta potential of nanoparticles, E) drug release profile, F) FTIR diagram and G) Cin absorption spectra.

3.2. Antioxidant activity

DPPH Scavenging activity of Cin was increased by increasing the concentration and reached 100 % at the concentration of 1000 µg/mL. In the Cin/PLGA NPs, an enhancement

of the antioxidant scavenging was observed with the increase in the nanoparticles concentration, but the slope of the graph was lower than that of the Cin (Fig. 2B,D).

3.3. Blood compatibility

Hemolysis of both Cin and Cin/PLGA NPs was concentration-dependent and was higher in Cin than Cin/PLGA NPs in all concentrations (Fig. 2A).

3.4. Cellular uptake of the nanoparticles

Fig. 2F is the fluorescent image of C6 cells which were incubated with Cin/PLGA NPs and Fig. 2G is DAPI staining of mentioned cells. According to Fig. 2H whenever nuclei were stained Cin/PLGA NPs were present, therefore Cin/PLGA NPs were uptaked by C6 cells.

3.5. Cytotoxicity of nanoparticles

It was observed that cellular toxicity of Cin and Cin/PLGA NPs in the C6 cell line was concentration and time-dependent. C6 viability was higher in Cin-treated cells in comparison with Cin/PLGA NPs treated cells at all concentrations (1µg/mL to 400 µg/mL) after 24 h, however, this is converted after 72 h which means Cin/PLGA NPs were more powerful than Cin to kill C6 cells (Fig. 2C,E).

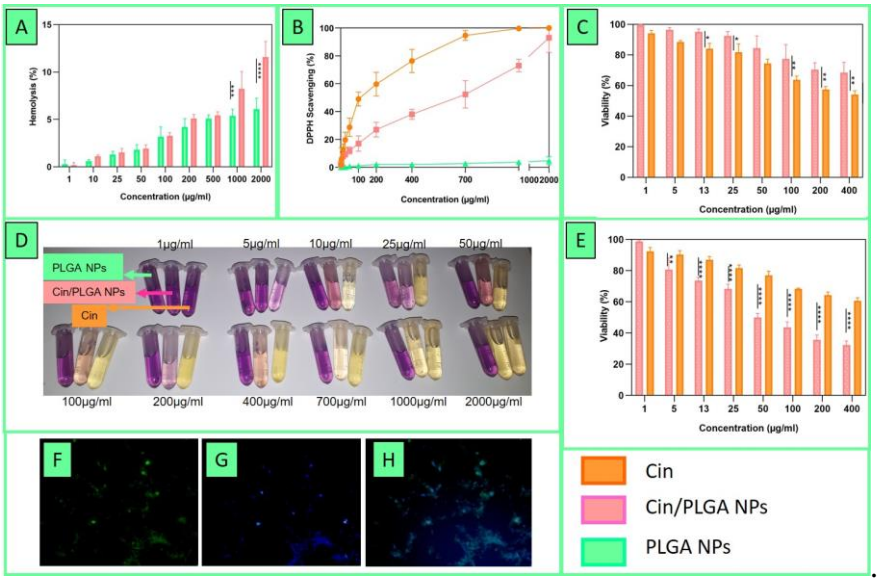


Figure 2. A)hemolysis diagram, B)antioxidant activity, C)MTT result after 24h, E)MTT results after 72 h, D)antioxidant activity, F,G,H) Cellular uptake images.

4. Discussion

Recently, there has been a great deal of focus on medicinal plants, including novel delivery systems [15]. In another study PLGA nanoparticles containing cinnamldehyde for antifungal activity were developed with a mean diameter of 130 nm and a zeta potential of -3 mV [16]. In addition with PLGA, other polymers such as PEG [4] and chitosan [17] were used for the delivery of cinnamon essential oil and cinnamon extract, respectively. Anti-tumor effect of Fe_3O_4 nanoparticle coated with cinnamaldeyde and FITC was seen on breast adenocarcinoma animal model [18]. In our study this is the first time that cinnamon extract was encapsulated in PLGA nanoparticles which is coated with PVA and poloxamer188.

5. Conclusions

In this study, PLGA nanoparticle containing cinnamon extract coated with PVA/poloxamer188 were prepared. The findings of this study indicate that Cin/PLGA NPs could be a promising adjuvant treatment for GBM. However, additional research is required, and we recommend using cinnamaldehyde instead of cinnamon extract for greater effectiveness and a smaller nanoparticle diameter.

Author Contributions: "Conceptualization, Masood.Khosravani and Mahdi.Adabi.; methodology, Fatemeh.Madani.; software, Fatemeh.Madani.; validation, Masood.Khosravani, Mahdi.Adabi.; formal analysis, Fatemeh.Madani.; investigation, Fatemeh.Madani.; resources, Fatemeh.Madani.; data curation, Fatemeh.Madani.; writing—original draft preparation, Fatemeh.Madani.; writing—review and editing Masood.Khosravani and Mahdi.Adabi.; visualization Mahdi.Adabi, supervision, Masood.Khosravani and Mahdi.Adabi.; project administration, Masood.Khosravani; funding acquisition, Masood.Khosravani All authors have read and agreed to the published version of the manuscript."

Funding: This research received no external funding.

Institutional Review Board Statement: All experiments were compliant with research ethic committee guidelines of the Tehran University of Medical Sciences **(**IR.TUMS.MEDICINE.REC.1400.425**).**

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: This research was supported by Tehran University of Medical Sciences, grant number 51516-148-1-1400"

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Haghi-Aminjan, H., et al., *The role of melatonin on chemotherapy-induced reproductive toxicity.* Journal of Pharmacy and Pharmacology, 2018. **70**(3): p. 291-306.
2. Singh, S., A. Sharma, and G.P. Robertson, *Realizing the Clinical Potential of Cancer Nanotechnology by Minimizing Toxicologic and Targeted Delivery ConcernsLimitations and Approaches of Cancer Nanotechnology.* Cancer research, 2012. **72**(22): p. 5663-5668.
3. Błaszczyk, N., A. Rosiak, and J. Kałużna-Czaplińska, *The potential role of cinnamon in human health.* Forests, 2021. **12**(5): p. 648.
4. Hemalatha, N. and K.R. Kumar, *Synthesis and characterization of PEG-cinnamon essential oil nanoparticles and their application as an insecticidal agent.* Journal of Advanced Scientific Research, 2021. **12**(03 Suppl 1): p. 142-148.
5. Ahmed, J., et al., *Anti-fungal bandages containing cinnamon extract.* International Wound Journal, 2019. **16**(3): p. 730-736.
6. Sartorius, T., et al., *Cinnamon extract improves insulin sensitivity in the brain and lowers liver fat in mouse models of obesity.* PloS one, 2014. **9**(3): p. e92358.
7. Frydman-Marom, A., et al., *Orally administrated cinnamon extract reduces β-amyloid oligomerization and corrects cognitive impairment in Alzheimer's disease animal models.* PloS one, 2011. **6**(1): p. e16564.
8. Shang, C., et al., *Beneficial effects of cinnamon and its extracts in the management of cardiovascular diseases and diabetes.* Food & Function, 2021. **12**(24): p. 12194-12220.
9. Assadollahi, V., et al., *The effect of aqueous cinnamon extract on the apoptotic process in acute myeloid leukemia HL-60 cells.* Advanced biomedical research, 2013. **2**.
10. Gopalakrishnan, S., et al., *Procyanidin-B2 enriched fraction of cinnamon acts as a proteasome inhibitor and anti-proliferative agent in human prostate cancer cells.* IUBMB life, 2018. **70**(5): p. 445-457.
11. Liu, Y., et al., *Targets and mechanism used by cinnamaldehyde, the main active ingredient in cinnamon, in the treatment of breast cancer.* Frontiers in Pharmacology, 2020. **11**: p. 582719.
12. Madani, F., et al., *Polymeric nanoparticles for drug delivery in glioblastoma: State of the art and future perspectives.* Journal of Controlled Release, 2022. **349**: p. 649-661.

13. Tayfeh-Ebrahimi, R., A. Amniattalab, and R. Mohammadi, *Evaluation of Effect of Biologically Synthesized Ethanolic Extract of Propolis-Loaded Poly (-Lactic-co-Glycolic Acid) Nanoparticles on Wound Healing in Diabetic Rats.* The International Journal of Lower Extremity Wounds, 2022: p. 15347346211073224.
14. Madani, F., et al., *Paclitaxel/methotrexate co-loaded PLGA nanoparticles in glioblastoma treatment: Formulation development and in vitro antitumor activity evaluation.* Life sciences, 2020. **256**: p. 117943.
15. Husni, P. and Z.M. Ramadhania, *Plant extract loaded nanoparticles.* Indonas. J. Pharm, 2021. **3**: p. 38-49.
16. Gursu, B.Y., İ. Dag, and G. Dikmen, *Antifungal and antibiofilm efficacy of cinnamaldehyde-loaded poly (DL-lactide-co-glycolide)(PLGA) nanoparticles against Candida albicans.* International Microbiology, 2021: p. 1-14.
17. Alghuthaymi, M.A., et al., *Green biosynthesized selenium nanoparticles by cinnamon extract and their antimicrobial activity and application as edible coatings with nano-chitosan.* Journal of Food Quality, 2021. **2021**: p. 1-10.
18. Shetty, V., et al., *Folate mediated targeted delivery of cinnamaldehyde loaded and FITC functionalized magnetic nanoparticles in breast cancer: in vitro, in vivo and pharmacokinetic studies.* New Journal of Chemistry, 2021. **45**(3): p. 1500-1515.