THE NOVEL POLYMERIC NANOCARRIER CONTAINING ASTRAGALIN DISPLAYS THE HEPATOPROTECTIVE EFFECT AGAINST CCl4-INDUCED LIVER INJURY IN VIVO

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Article History: **Received**: 18.06.2022 **Revised**: 17.07.2022 **Accepted**: 09.08.2022

Abstract: A flavonoid with therapeutic qualities, astragalin is one of the least researched and has been utilized in Southeast Asian traditional medicine. The protective effects of astragalin-loaded polymeric nanoparticles (AST-NPs) were formulated, optimized using central composite factorial design (CCD), and evaluated Hepatoprotective activity on CCl_4 -induced liver damage in experimental rats. AST-NPs were prepared by the dialysis method.CCD was employed to study the influence of formulation factors, polymer concentration, aqueous organic phase ratio, and process parameter stirring time on dependent physicochemical characteristics, particle size, zeta potential, and percentage entrapment efficiency (%EE) of the drugs. In-vitro release studies, stability tests, and an evaluation of the formulation's hepatoprotective activity were all conducted on the optimized formulation. Fourier transmission infrared (FT-IR), differential scanning calorimetry (DSC), drug loading, entrapment effectiveness, particle size, zeta potential, and in vitro investigations were used to characterize the produced NPs. There was no evidence of a drug-polymer interaction, according to FT-IR and DSC experiments. The improved NPs show stability. The zeta potential of -25 mV, the %EE of 89%, and the mean PS of 118 nm of optimized PNPs all showed spherical and porous surfaces.

Keywords: Astragalin, Central Composite Design, Encapsulation Efficacy, Hepatotoxicity, Particle Size, Polymer Nanoparticle.

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DOI: 10.31838/ecb/2022.11.06.001

INTRODUCTION

The metabolism and detoxification of substances that enter the body and may induce hepatic damage, which can result in life-threatening disorders, are critical functions of the liver[1]. Liver disorders have traditionally been treated using natural remedies. As a result, the recent importance of plant-based herbal medicines' preventive benefits against drug-induced toxicity has increased[2]. A flavonoid known as astragalin (kaempferol-3-O-glucoside) is obtained from the leaves of persimmon, Rosa agrestis, or green tea seeds. *Astragalin* has a wide range of pharmacological properties, including antioxidative, anti-inflammatory, and anticancer activity. It can also improve the effects of apoptosis, according to several preclinical investigations[3,4,5]. The objective of this study was to assess astragalin's hepatoprotective properties.

When living creatures are exposed to carbon tetrachloride (CCl4), a xenobiotic that is dumped into the water as waste from various industries, it can cause hepatotoxicity[6]. Due to its features including enhanced bioavailability, reduced toxicity, and increased biodegradability, polymeric nanoparticles have been used for a variety of medicinal reasons [7,8]. Because of their small size, they are known to interact with biological systems without difficulty[9]. Proteins (like gelatin and milk proteins), polysaccharides (like chitosan, sodium alginate, and starch), and synthetic polymers (like poly (d,l-lactide), poly (lactic acid), poly (d,l-glycolide), poly (lactide-co-glycolide), and poly (cyanoacrylate) PCA) can all be used to make biodegradable polymeric nanoparticles [10].

Astragalin-loaded polymeric nanoparticles were created by the dialysis method in the present work. There is evidence that astragalin has hepatoprotective effects. However, there are no reports on astragalin's or its nano formulation's hepatoprotective properties. As a result, we decided to investigate the hepatoprotective properties of astragalin-loaded polymeric nanoparticles in a rat model of CCl_4 intoxication.

MATERIALS AND METHODS

Chemicals

Astragalin, Polylactic acid (PLA), Dimethyl sulfoxide(DMSO) were purchased from Sigma Aldrich, India and, all other HPLC grade solvents were obtained from Thermo Scientific, India. Swiss albino rats were purchased from the Central animal house facility, Tamilnadu Veterinary, and Animal Sciences

University, Chennai, India. All animal experimentation protocol was reviewed and approved by the Institutional Animal Ethics Committee, K.L.R Pharmacy College, Paloncha India.

Formulation Of Astragalin-Loaded Polymeric Nanoparticles

A dialysis method was used to create the astragalin-loaded nanoparticles. After being dissolved in 1 mL of DMSO, astragalin (5 mg) and PLA (50 mg) were added dropwise to 25 mL of water while being stirred. The mixture was mixed for an additional 30 minutes at room temperature before being dialyzed for 24 hours using a 7 kDa dialysis bag and distilled water. A 0.45 m filter was used for filtration, and the unentrapped astragalin was then freeze-dried[11,12].

Characterization Of Nanoparticle

Particle Size Measurement

Malvern Zetasizer was used to measure the average particle size (z-average) and polydispersity index (PDI) of the produced nanoparticles (Malvern, Worcestershire, UK). By diluting the NPs suspension to 1/50 v/vin HPLC water, a three-way particle size examination was carried out. The PDI value represents the distribution of nanoparticles by size in a given sample. A greater PDI value shows the distribution of NPs with a varied size range, which can lead to aggregation formation, low particle suspension stability, and low homogeneity.

Zeta Potential

Malvern Zetasizer was used to evaluate the zeta potential after the nanoparticle suspension had been diluted by 50 times with HPLC water (Malvern, Worcestershire, UK). Zeta potential, which measures the stability of nanoparticles in suspension, represents the surface charge on the particles.

SEM Analysis

The samples for SEM were put on metal stubs, and a Hitachi S4800 Field Emission SEM was used to investigate the particles' surface and surface morphology (Hitachi, Gaithersburg, MD, USA). The analytical settings included a vacuum of 40 Pascals, a working distance of 13.5 mm, and an accelerating voltage of 10 KeV.

Differential Scanning Calorimetry (DSC) Analysis

Pure astragalin, PLGA, physical mixtures, and astragalin NPs were all analyzed using an ADSC (Shimadzu DSC-60, Columbia, MD, USA). The sample (3-5 mg) for DSC analysis was sealed loosely in an aluminum pan and heated at a rate of 10°C/min from room temperature to 300°C while being evacuated of nitrogen.

Fourier Transform Infrared (FTIR) Analysis

FTIR analysis was performed using a Perkin Elmer BX II to investigate the chemical interaction between the medication and the polymer (PerkinElmer, Massachusetts, USA). The samples were scanned between 400 and 4000 cm^{-1} in the IR region.

Encapsulation Efficiency

The drug loading efficiency and encapsulation efficiency (EE) were calculated using the following formulae.

$$DL = \frac{Amount\ of\ AST\ in\ NPs}{Amount\ of\ AST - Loaded\ NPs} \times 100$$

$$EE = \frac{Amount\ of\ AST\ in\ NPs}{Amount\ of\ AST\ for\ loading} \times 100$$

A C18 column (5 µm, 4.6250 mm) was used in a high-performance liquid chromatography (HPLC) system to measure the concentration of astragalin. Methyl alcohol, water, and acetonitrile were combined and supplied as the mobile phase at a flow rate of 1.0 mL/min. The wavelength was tuned at 227 nm, and the injection volume was 20 L.

In-Vitro Release Study

The dialysis bag diffusion technique was used to assess the in vitro release of astragalin-loaded nanoparticles. As the dissolution media for the in vitro release test, 100 milliliters of phosphate buffer with a pH adjustment to 7.4 were put into a tightly closed glass jar. A dialysis bag (molecular weight cutoff 5000–10,000) was used to contain astragalin NP (5 ml), which was then put inside the glass vessel. The vessels were put into an incubator shaker (IncubatorShakerZHWY-200B, Shanghai Zhicheng Analysis Instrument Company, China) and shaken horizontally at 37°C and 100 strokes per minute. At regular intervals, the sample (1 ml) was removed from the system and passed through a 0.45 m hydrophilic filter membrane. The drug content was assessed using the aforementioned HPLC technique. As a check, the diffusion profile of a drug suspension via a dialysis bag was investigated. The purified medicines suspension was made by dispersing 1 mg of astragalin (5 mg/ml) in 4 mg of double-distilled water. Three duplicates of each experiment were carried out.

Stability Studies

According to ICH guidelines, optimized AST-PLA-NPs were put through a six-month stability test at 2-8°C, 25°C, and 60% RH. In a glass vial with an amber hue, samples are kept. At a predetermined time interval, the samples were examined for the size of particles, zeta potential, assay, and drug release.

In Vivo Hepatoprotective Effect

A model of liver injury caused by carbon tetrachloride (CCl_4) was utilized to assess the hepatoprotective efficacy. Four groups of six Wistar albino rats were formed from the total population. Group 1 acted as the standard control and received 1 cc of normal saline daily for 9 days. On days 3, 6, 9, and 12, Group 2 was given CCl_4 (dissolved in three times its volume of olive oil) intraperitoneally at a dose of 0.7 ml/kg as a hazardous control. For two weeks, Group 3 was given an oral astragalin medication solution (100 mg/kg). For two weeks, Group 4 got an equal dose of astragalin nanoparticles orally every day. Except for the normal control group, all groups received CCl4 on days 1, 3, 6, and 12 of the study. On the final day of the trial, the animals were sedated, and blood was drawn by heart puncture. Centrifugation was used to separate the plasma from the blood samples for 15 minutes at 3000 rpm. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT) levels in the plasma were used to measure the hepatoprotective efficacy. They were then histopathologically examined for their livers. After the investigation, the rats were slaughtered, the liver was carefully removed, kept in formalin solution, and liver slices were produced. Additionally, the rats' body weights were tracked[13-16].

RESULTS AND DISCUSSION

Particle Size, Zeta Potential, And Sem Measurement

Astragalin-loaded polymeric nanoparticles had an average particle size of 121 2.6 nm (Figure 1). The same was determined to have a zeta potential of -25 1.4 mV (Figure 2), which is high enough to produce a stable pharmaceutical formulation. According to a paper, nanoparticles with a significant negative or positive zeta potential value are less likely to aggregate and

show strong stability by growing in size[17]. Figure 3 shows photographs taken using an SEM to provide details on the morphology of the ideal astragalin-loaded polymeric nanoparticles. The form of the optimized nanoparticles is spherical.

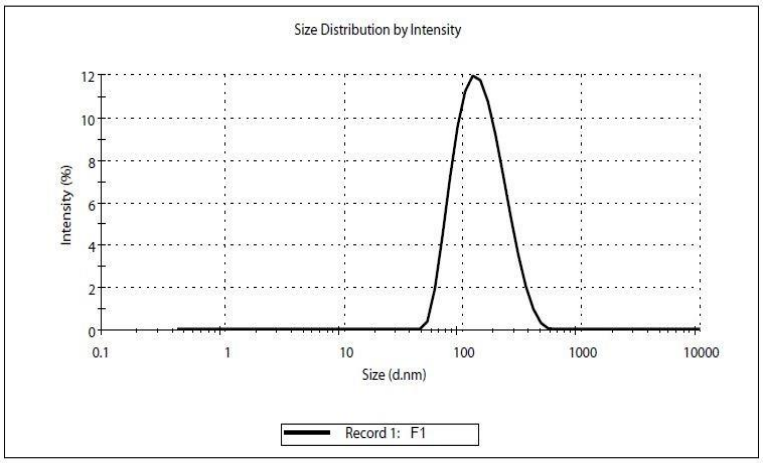

Figure 1. Particle size of optimized astragalin-loaded polymeric nanoparticle

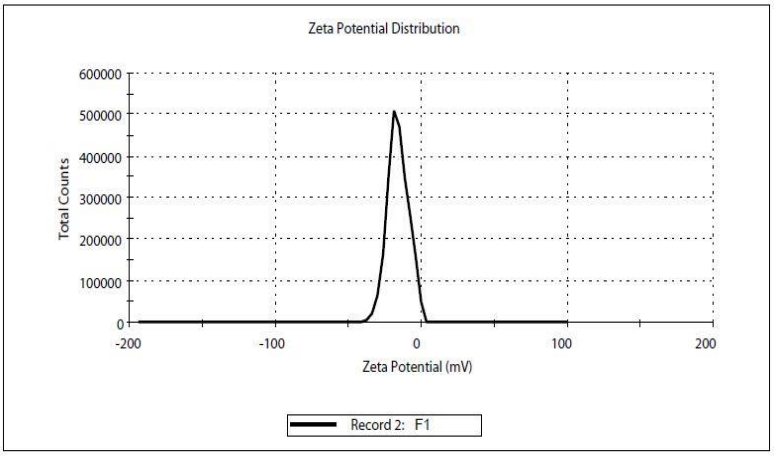

Figure 2. Zeta potential of optimized astragalin-loaded polymeric nanoparticle

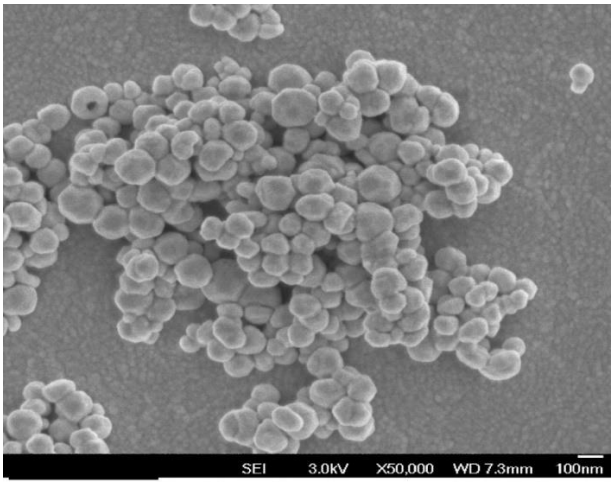

Figure 3. SEM image of optimized astragalin-loaded polymeric nanoparticle

Differential Scanning Calorimetry (DSC) analysis

A thermal analytical technique called differential scanning calorimetry (DSC) examines the energy a sample absorbs or

emits as a function of temperature[18]. DSC was used to examine astragalin's thermal properties. The endothermic peak of pure astragalin, which is sharp and corresponds to the melting point of 202°C, is visible. At 165°C, the PLA thermogram displayed a clear endothermic peak (Figure 4). Astragalin's DSC thermogram was compared to the DSC thermogram of the astragalin and polymer mixture utilized in the formulation, and it was determined that there shouldn't be any interference between the peaks of the drug and polymer. The DSC of the drug sample and polymer mixture was discovered to be within the prescribed range.

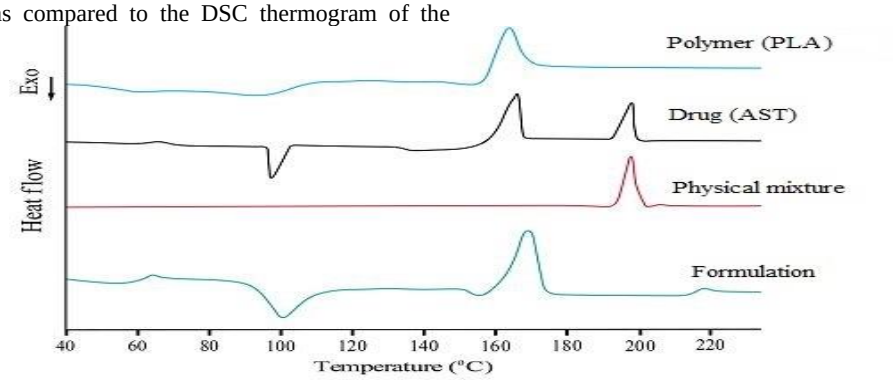

Figure 4. DSC of astragalin, PLA, physical mixture, and nano formulation

Fourier Transmission Infrared spectroscopy (FTIR) analysis

FTIR analysis is used to study the interactions between astragalin and the polymer PLA used in the formulation. The infrared spectra of astragalin, the polymer used, their physical mixture, and the formulation of the same were shown in figure 5. The IR of the mixture of drug sample and PLA were found to be within the specified range. Hence there is no interaction between the drug sample and the polymer likely to be used in the formulation and can be used in the formulation[19]. Astragalin procured their entire characteristic peak in the physical mixture. That is a significant peak of 806-1653 was retained in the physical mixture. The frequency vibration of C=C stretching of AST at 1653 and 1613 cm^{-1} shifted to 1686 and 1628 cm^{-1}, respectively. The out-of-plane C−H bending at 806 cm^{-1} in AST shifted to 795 cm^{-1} in the complex. Other minor changes (shifting/intensity variation) were also observed in AST at 1362 cm^{-1} (in-plane OH bend), 1208 cm^{-1} (C−O stretching),1508 and 1448 cm^{-1} (C−C stretching (in the ring)) based on FTIR spectra investigation no chemical interaction were observed between drug and polymer.

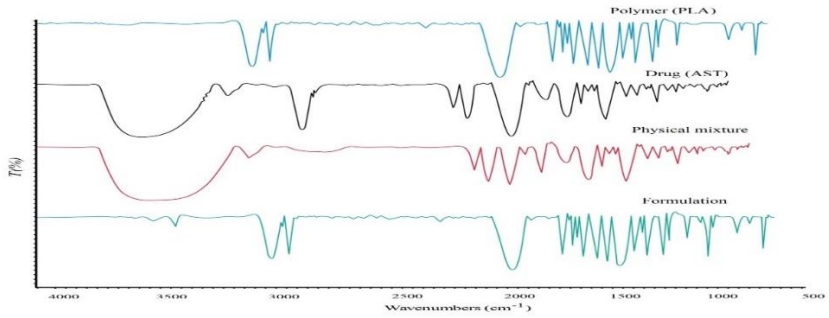

Figure 5. FTIR of astragalin, PLA, physical mixture, and nano formulation

The in vitro drug release study

The capacity of an anticancer formulation to maintain stability at physiological pH while releasing its payload at the tumor site is its most desired property[20]. Analyses of drug in-vitro release were carried out utilizing the dialysis bag technique. The pH of a Phosphate Buffer was adjusted to 7.4 before astragalin-loaded PLA nanoparticles (5 mg) were introduced and swirled magnetically for 32 hours. At 30, 60, 90, 120, 150, 180, 210, 240, and 270 minutes, the amount of astragalin released from the nanoparticles was measured. Astragalin was quickly released in phosphate buffer solution, with a cumulative release rate of about 94% in 120 minutes, according to the data. The astragalin-loaded PLA nanoparticles produced a sustained-release effect and were rapidly released, releasing 65% of their total amount in the first 120 minutes before releasing steadily and slowly after that. The AST-PLA-NPs in vitro showed a clear sustained-release impact in comparison to AST.

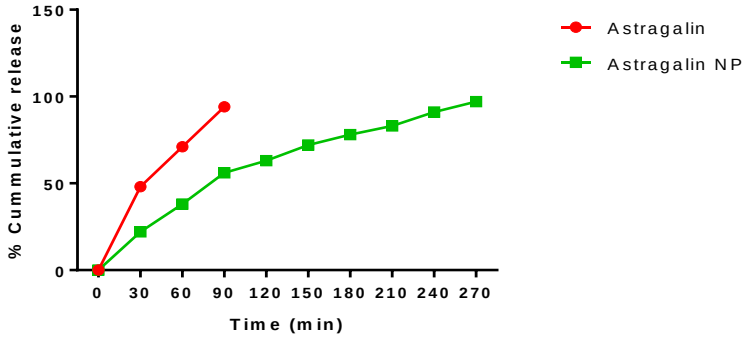

Figure 6: Cumulative Percentage drug release of pure drug astragalin and astragalin-loaded polymeric nanoparticles in PBS (pH 7.4)

Stability of astragalin loaded polymeric nanoparticles

Particle size, Zeta potential, assay, and in vitro drug release for the formulation stored at 2- 8°C and 25°C for 6 months did not show any discernible differences. Polymeric nanoparticles with astragalin were discovered to be stable.

Table 1: Stability Results of optimized nanoparticle

Storage Condition		**25°C/60% RH**		**2-8 °C**		
Time Point	**Initial**	**3 Month**	**6 Month**	**1 Month**	**3 Month**	**6 Month**
Description	Colloidal Dispersion	No Change	No Change	No Change	No Change	No Change
Particle Size (nm)	121± 2.6	128± 2.3	132± 1.9	120± 2.1	124± 2.2	125± 1.8
Zeta Potential (mV)	-25± 1.4	-23± 1.2	-22± 1.1	-25± 1.2	-25± 1.1	-23± 0.9
Assay	99%± 0.9	98.50%± 0.7	99.20%± 0.6	100%± 0.8	99%± 0.5	99%± 0.8
In vitro release at 210 minutes	94%± 0.7	92%± 0.5	95%± 0.8	93%± 0.9	95%± 0.6	97%± 0.7

Data represent mean±SD, *n = 3*

In Vivo Hepatoprotective Effect

Data on hepatoprotective efficacy are shown in Table 2. The animals' SGPT and SGOT activities significantly increased after receiving CCl_4, indicating higher toxicity, although this was lessened in the mice given astragalin nanoparticle treatment. For both astragalin nanoparticle and astragalin solution, the decrease in toxicity was statistically significant at p 0.001. The higher levels of SGOT and SGPT were reversed by the astragalin nanoparticles, though.

Table 2. Effect of astragalin nanoformulation on enzyme levels in rats with carbon tetrachloride (CCl4) induced hepatotoxicity

Treatment group	**Initial body weight (g)**	**Body weight after 9 days (g)**	**SGPT (U/L)**	**SGOT (U/L)**
Control	159±6	175±3	10.6±1.8	29.9±2.2
CCl_4	164±8	144±9	72.7±2.6	89.4±1.9
Astragalin solution	168±5	176±4***	41.2±6.2***	61.1±2.6***
Astragalin nanoparticle	169±3	180±2***	15.69±2.8***	37.8±2.4***

***P<0.001, **P<0.01, *P<0.05 compared with CCl_4 group

CONCLUSION

In the present investigation, an effort was made to construct and optimize astragalin-loaded polymeric nanoparticles using an experimental design method to create better drug delivery systems. Particle size, zeta potential, and encapsulation effectiveness of polymeric nanoparticles are affected by polymer concentration, stirring time, and the ratio of aqueous to organic phases. The 50 mg/ml polymer concentration, 60 minutes of stirring, and a 5:1 aqueous to organic phase ratio was used to create the optimal formulation. The particle size of the final formulation is 121 nm, the Zeta potential is -25 mV, and encapsulation efficiency is 89%. The physical and chemical stability of the polymeric nano formulation is established by stability experiments on the improved formulation. The present study found that optimized astragalin nanoparticles had

hepatoprotective action against liver damage caused by CCl4, although more in-depth research is needed. The mechanism underlying the drug's antifibrotic action is not yet understood, but it will be thoroughly investigated in the future.

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