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Research Article

Preparation and characterisation of nanoparticles containing Andrographolide prepared by solvent evaporation method

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ABSTRACT

This study was focused on preparation of AND-SLNs using solvent evaporation method for liver targeting. Characterization of andrographolide was accomplished by using various methods including spectral, chemical, thermal and chromatographic principles. The prepared AND-SLNs were characterized using Zeta, PDI and transmission electron microscopy. The drug-loaded AND-SLNs encapsulation efficiency and behaviour of *in-vitro* drug release were studied using UV spectroscopy. The *in-vitro* release study of AND-SLNs compare with the standard drug Silymarin. The release of active moiety andrographolide from the solid lipid nanoparticles showed a biphasic pattern, burst release initial pattern and secondly maintain sustained release. Andrographolide was administered at 500 mg/kg by oral gavages to test its protective effect on hepatic injury biochemically and histopathologically in the blood/liver and liver respectively. In conclusion, andrographolide produced a protective effect against CCl_4-induced liver damage. Our study suggests that Andrographolide may be used as a hepato-protective agent against toxic effects caused by CCl_4 and other chemical agents in the liver.

Key words: Nanoparticles, Andrographolide and Hepatoprotection

Introduction

Nanotechnology mainly deals with the Nanoparticle having a size of 1–100 nm in one dimension used significantly concerning medicinal chemistry, atomic physics, and all other known fields [1]. Since nanoparticles (NP) offer sustained-release action and therapeutic targeting, drug delivery to hepatocytes via NP is particularly interesting [2]. One method for selective medication delivery that shows promise is receptor-mediated drug targeting [3]. To improve selectivity toward liver cells, an effective strategy is to design nano-sized carrier to realize liver-targeted delivery [4]. Recently, nanoparticles have been proved to have the advantages in drug delivery with low system toxicity [5-7]. Many nano-sized drug delivery systems, such as natural and synthetic polymer nanoparticles, metal nanoparticles, and polymer-drug conjugates, have been investigated for delivery of hepatostimulant and hepatoprotective drugs [8]. The nano-vehicles basing on phosphoethanolamine-polyethylene glycol polymers (PEG-PE) represent a promising nanoparticles delivery system owing to biocompatibility, prolonged circulation, and accumulation in liver by the enhanced permeability and retention (EPR) effect [9]. In the past decade, many efforts have been made to prepare liver-targeting nano-carriers [10].

Andrographis paniculata (Burm. F) the herbaceous plant nees, which belongs to Acanthaceae family, and frequently referred to as the "king of bitters." Southeast Asia, India tropical and subtropical Asia is its native regions. This plant is also known as "Kalmegh" in our country India, some different name in different country [11]. The plant's extracts and andrographolide shows various Pharmacological properties include immunostimulatory [12], antiviral [13], and antibacterial actions [14]. Primary effective chemical ingredient, andrographolide has shows a wide or broad spectrum biological actions, including various effects [15]. A. paniculata contains andrographolide, a significant bioactive phytoconstituent, in a variety of tissues, but especially leaves. The chemical name of andrographolide is 3α, 14, 15, 18-tetrahydroxy-5β, 9βH, 10α-labda-8, 12-dien-16-oic acid γ-lactone and its molecular formula and weight are $C_{20}H_{30}O_5$ and 350.4 (C 68.54%, H 8.63%, and O 22.83%). These are the numerous methods that have been utilized to investigate the structure of [16–20]. Active moiety of andrographolide is freely soluble in various organic solvents, in comparison of water. Over a three-month period, crystalline form of andrographolide was said to be extremely stable [21]. Different types of liver ailments continue to be a critical health issue and a leading cause of death. Modern medicine lacks effective hepatoprotective medications, so controlling many liver problems using herbs and plants is essential [22]. There is a wide variety of compounds that exhibit powerful hepatoprotective efficacy, according to a substantial body of literature on hepatoprotective action of drugs from natural sources. A. paniculata has been used for many years in Indian medical systems as a hepatoprotective and hepatostimulant drug [23]. Moreover, andrographolide commonly used in various polyherbal hepatoprotective formulations [24]. it has been shown to be better effective in hepatitis B treatment [25]. According to literature review, andrographolide was reduces the liver problems associated with various drugs and chemicals [26]. It was find out that active compound andrographolide shows a more powerful in comparison of standard drug silymarin against the paracetamol induced hepatic disorders. Silymarin and andrographolide was shows similar effect against the treatment of ethanol-induced hepatotoxicity [27]. Single dose of

andrographolide and extract of andrographolide is more effective against the CCl_4 induced toxicity and andrographolide have been investigated in context of hepatic microsomal lipid peroxidation caused by carbon tetrachloride (CCl_4) [28]. A. paniculata extract demonstrated hepatoprotective properties in line with its traditional uses and pharmacology [29].

Liver is a largest and critical organ that plays an important role in the conjugation and detoxification of numerous medications [30]. Xenobiotics and infections tend to compromise its activity. Excessive exposure to xenobiotics can cause cirrhosis or cancer if not treated. Currently, millions of people suffer from liver impairment caused by alcohol, drugs, and diseases. Both Acute and chronic types of liver disorders remain a significant global health concern [31]. Drug paracetamol [32], carbon tetrachloride (CCl_4) [33], nitrosamines, and polycyclic aromatic hydrocarbons and some other types of chemicals cause severe liver damage. Now newer and safer hepatoprotective medicines are needed to replace present ones. Modern medicine has limited alternatives due to their instability and inefficiency [34]. Recent research indicates that exposure to liver-toxic substances, ionizing radiations, environmental contaminants, and drugs might result in the formation of oxygen-free radicals like superoxide anion radical (2^{-}) and hydroxyl radical ($OH^{\bullet}$), leading to hepatotoxicity [35]. CCl_4 is a popular chemical solvent in manufacturing industry. This hepatotoxin is the most extensively studied animal model of xenobiotic-induced free radical-mediated hepatotoxicity [36]. CCl_4 produces liver damage through many routes [37]. CCl_4 is considered to cause hepatotoxicity by enhanced lipid peroxidation caused by free radical production [38]. CCl_4 also activates immune systems by bringing inflammatory cells to the site of harm. Immune cells may emit pro-inflammatory cytokines including TNF-α and IL-6, leading to increased hepatotoxicity through recurrent inflammation.

Material and Method

Drug and Chemical

The drug andrographolide was purchased from Ambe NS Agro Product (with a purity ≥ 98%). Stearic acid and Pluronic were obtained from Sigma-Aldrich, India, a well-known distributor of analytical-grade chemicals and laboratory supplies. Sigma-Aldrich is recognized for its stringent quality control measures and adherence to international standards, ensuring the reliability and purity of the chemicals procured for research and experimentation purposes. All other chemicals and solvents used in the experimental work were of analytical grade.

Animal

After animal ethical approval 1204/PO/Re/S/08/CPCSEA/23-03 in this study male Wistar albino rats weighing about 200–250g (10–12 weeks old) were used. Experimental specimens were obtained from the Animal Ethics committee, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University. They were kept in perfect laboratory settings with 12-hour light/dark cycle, 45–55% RH and 23–25°C temperature. During the entire experiment, they were fed a regular pellet diet and had access to water at all time. Every experiment was conducted in compliance with the standards set forth by Swami Vivekanand Subharti University's animal care and use committee.

Method preparation of Solid Lipid Nanoparticles

The solid lipid nanoparticles formulations were prepared by using the solvent evaporation method. Two factors were selected (X1: lipid concentration and X2: surfactant concentration)

and the effect on three responses (Y1: Particle size, Y2: % PDI and Y3: entrapment efficiency) was determined. Briefly, the organic phase was prepared by dissolving andrographolide (100 mg) and stearic acid (100 to 200 mg) in 10 mL of methanol. The organic phase was dispersed drop by drop in the 20 mL aqueous phase containing varying concentration (75 to 150 mg) of Pluronic as surfactant. The emulsion was stirred at 10000 rpm. Finally, the temperature of the system was elevated to 75°C. The developed solid lipid nanoparticles were separated by freeze-drying.

Melting Point Determination

Melting point of drug andrographolide was determined by melting point apparatus using capillary method. A capillary tube was taken and its one end was sealed. The open end of the capillary tube was filled with dry drug powder. Then the capillary tube was placed in the melting point apparatus. The temperature at which sample powder starts to melt was noted with the help of thermometer and compared with literature value or certificate of analysis of drug. It was observed the melting point of andrographolide sample was found to be 235°C. It is matched to the reported melting point of andrographolide.

FTIR spectroscopy

Fourier transforms infrared absorption spectrum of the drug was determined. The drug sample (10mg) should be grounded and reduced the particle size. Otherwise, large particle scatter the infrared beam and affect the slope baseline of spectrum [39]. Add the powder uniformly to the 7mm collar. Put the die together with the powder in to the quick handy press. Place the collar jointly with the pellet in to the sample holder. Place the drug in the sample holder of FTIR instrumentation (Bruker model Alpha). The spectrum was recorded using the software IR solution in the range of 400- 4000 cm^{-1}.

Differential Scanning Calorimetry (DSC)

Thermal analysis of the procured sample was determined with the help of differential scanning calorimetry (DSC) instrument model make Perkin Elmer. The (20mg) drug sample was weighed and transferred to DSC pan and crippled using hydraulic press. The sample pan was placed in the DSC sample holder port and instrument allowed to run through computer operated software (Pyris-6 Version 4.0) in the temperature range 10°C/min from (30°C to 400°C) during the measurement, and the sample cell was continuously purged with nitrogen at a flow rate of 10 ml/min heating rate [40].

Spectrophotometry

UV spectrum of drug sample was measured using UV-Spectrophotometer (Shimadzu Model No. FTIR8100). Sample (10 mg) was weighed and transferred to 100 ml volumetric flask and diluted with methanol suitably. Then stock solution was diluted to 10 times and UV spectrum was scanned in the range of 200-400nm. UV spectrum of the procured sample of andrographolide showed a maximum absorption at maximum 236 nm which is identical with reported value in certificate of analysis [41].

Analytical Methodology (**Spectrophotometric method)**

Accurately weight the quantity (10.0mg) of drug sample on analytical balance (Shimadzu Model No. AY220), and was dissolved in 10 ml methanol taken in amber color volumetric flask covered with black paper. It was marked as the primary stock solution of drug having concentration (1000 µg/ml). 1.0 ml of this primary stock solution was taken in another volumetric container diluted to 10.0 ml with methanol to obtain conc. of 100 µg/ml solution.

This solution taken in volumetric container was previously wrapped in black paper sheet. It was denoted as secondary stock solution. Further, ten different aliquots from secondary stock solution was taken in different test tubes (measuring 1, 2, 3, 4 & 5ml) and diluted with methanol to get the concentration of 1, 2, 3, 4, 5µg/ml). The absorbance of resulting solutions was measured at λ max 236 nm by using UV- Vis spectrophotometer instrumentation Shimadzu Model No. FTIR8100 keeping methanol solution used as a blank. The entire procedure was repeated thrice. A graph was plotted between absorption vs. concentration value of resulting solution in excel sheet of MS Excel software and statistical parameters were determined as correlation coefficient and regression line [42].

Table 1: Calibration curve of andrographolide in methanol

S. No.	Concentration (µg/ml)	Absorbance (nm)
1	1.00	0.036 ± 0.004
2	2.00	0.0848 ± 0.006
3	3.00	0.1278 ± 0.007
4	4.00	0.184 ± 0.006
5	5.00	0.2402 ± 0.009

Particle size, polydispersity index and zeta potential

Zetasizer (Zetasizer 3000 HAS, Malvern Instruments Ltd, Worcestershire, UK) was used to measure the developed nanoparticles' particle size, polydispersity index, and zeta potential. Particle size and PDI measurements were made using polystyrene cuvettes containing filtered deionized water, which were then diluted and examined at a fixed 90-degree angle. Using a laser-based multiple angle particle electrophoresis analyzer, the zeta potential of the nanoparticles was evaluated. The nanoparticles were dispersed in distilled water (pH 6.8) and then put the sample in an electrophoretic cell with an electric field of 15.24 V/cm [43].

Surface morphology

The morphology of solid lipid nanoparticles was observed using transmission electron microscopy (JEM-2010 HR, JEOL, Japan). On a copper grid, a single drop of the nanoparticle suspension was stained for two minutes using a 2% phosphotungstic acid solution. The grid was studied under an electron microscope after being let to dry at room temperature.

Entrapment Efficiency

The andrographolide-loaded nanopaticles were extracted from suspension using 15 minutes of ultracentrifugation at 50,000 rpm. A UV-Vis spectrophotometer (Shimadzu 1800, Tokyo, Japan) was used to measure the amount of free andrographolide in the supernatant at a wavelength of 236 nm [44]. Using the following formulas were used to determine the loading capacity (LC) and encapsulation efficiency (EE) of the andrographolide nanoparticles:

$$EE\ (\%) = (X\text{-}Y)/X \times 100$$

$$LC\ (\%) = (X\text{-}Y)/Z \times 100$$

Where: *X* means amount of total drug andrographolide, *Y* stand the total free amount of drug andrographolide present in supernatant, and *Z* means weight of andrographolide nanoparticles.

In-vitro release study

A solution of phosphate buffer saline (PBS) (pH 7.4) was used to assess the andrographolide in vitro release profiles of the nanoparticles. The lyophilized nanoparticles of andrographolide 10milligrams were distributed in 10ml of PBS (pH 7.4), and the particle Next, suspension was put inside dialysis bag 12 000 MW threshold). Tied the bag was submerged in 100ml of PBS solution. The entire system was shielded from light and kept at 37 ± 2°C with constant magnetic stirring at 100 rpm. 1ml sample diffusion medium was collected at the proper time intervals and immediately replaced with 1ml fresh PBS (pH 7.4). The released amount of andrographolide was determined by using UV Spectrophotometer at 236 nm [45]

Result and Discussion

Preparation of AND-SLNs

The solid lipid nanoparticles were prepared by using the solvent evaporation method [46]. Two selected factors (X1: lipid concentration and X2: surfactant concentration) were shown in (**Table 2**) Briefly, the organic phase was prepared by dissolving andrographolide (100 mg) and stearic acid (100 to 200 mg) in 10 mL of methanol. The organic phase was dispersed drop by drop in the 20 mL aqueous phase containing varying concentration (75 to 150 mg) of Pluronic as surfactant. The emulsion was stirred at 10000 rpm. Finally, the temperature of the system was elevated at temperature 75°C. The developed solid lipid nanoparticles were separated by freeze-drying. The effect on three responses (Y1: Particle size, Y2: % PDI and Y3: entrapment efficiency) was determined. The present drug loaded formulation was selected on the basis of maximum drug entrapment efficiency of AND-SLNs was found to be (**Table 3**)

Table 2: Preparation of SLNs by using different ratio of lipid and surfactant concentration

Run	X1: Lipid concentration (mg)	X2: Surfactant concentration (mg)
1.	80.2893	112.5
2.	150	112.5
3.	200.711	112.5

Characterization of Drug Sample and AND-Nanoparticles

In the preformulation studies of this research work, identity of the procured drug sample and excipient employed in the development of nanoparticles system was established. Characterization of andrographolide was accomplished by using various methods including spectral, chemical, thermal and chromatographic principles.

Spectral, Chemical and Thermal Characterization

U.V. spectrum of drug sample was measured in methanol is given in **Figure 1**. It is revealed from the spectrum that sample has λmax value at 236 nm which is almost identical to that of spectrum reported in the literature [41]. To devise the analytical methodology of drug sample, U.V. spectrum of drug sample in solvents were measured. In order to quantify the amount of andrographolide in solutions, an analytical method was drawn on the basis of U.V.

Spectrophotometric principles. Methods of drug estimation in different solvents and its λmax values, r^2 values (≈0.997), entire absorbance values etc. are given in the **Table 1.** Standard curve/plot of andrographolide prepared in methanol is given in the **Figure 2**. All of these methods follow Beer's Lamberts law and demonstrated that drug absorbance follow linear range of drug concentration (1-5ug/mL). Standard solutions of drug were also observed stable at room temperature in bench top stability [42].

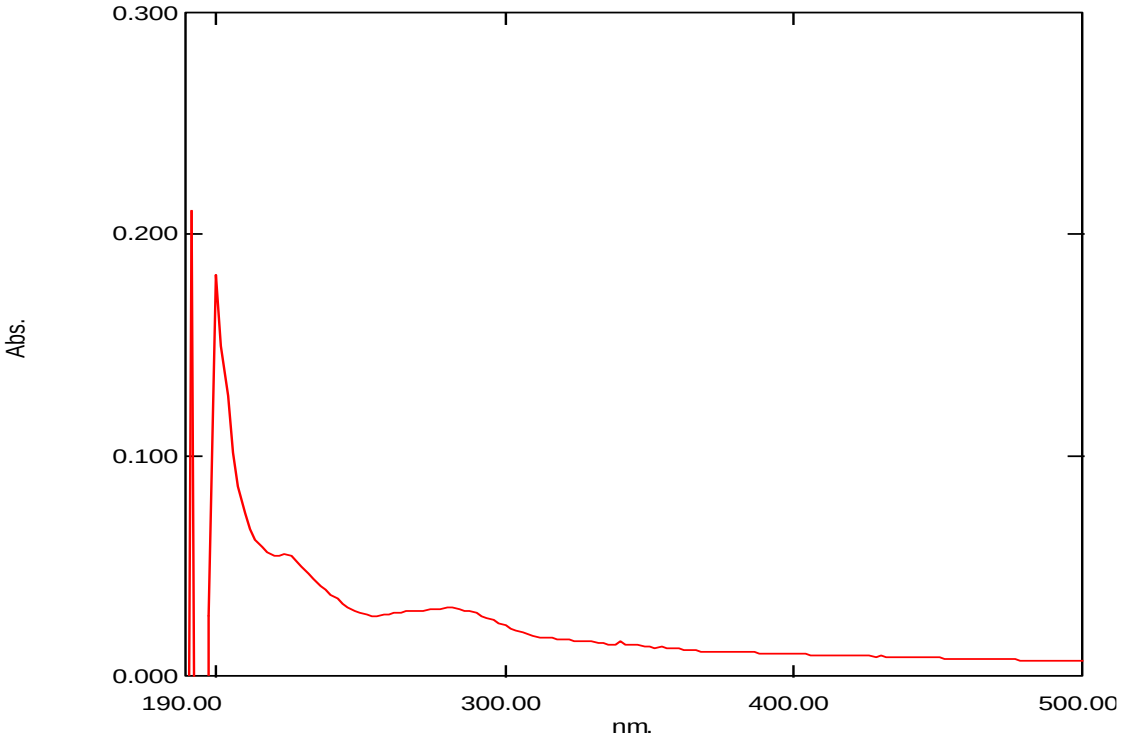

Figure 1: Representation of UV spectrum of Andrographolide in methanol (sample)

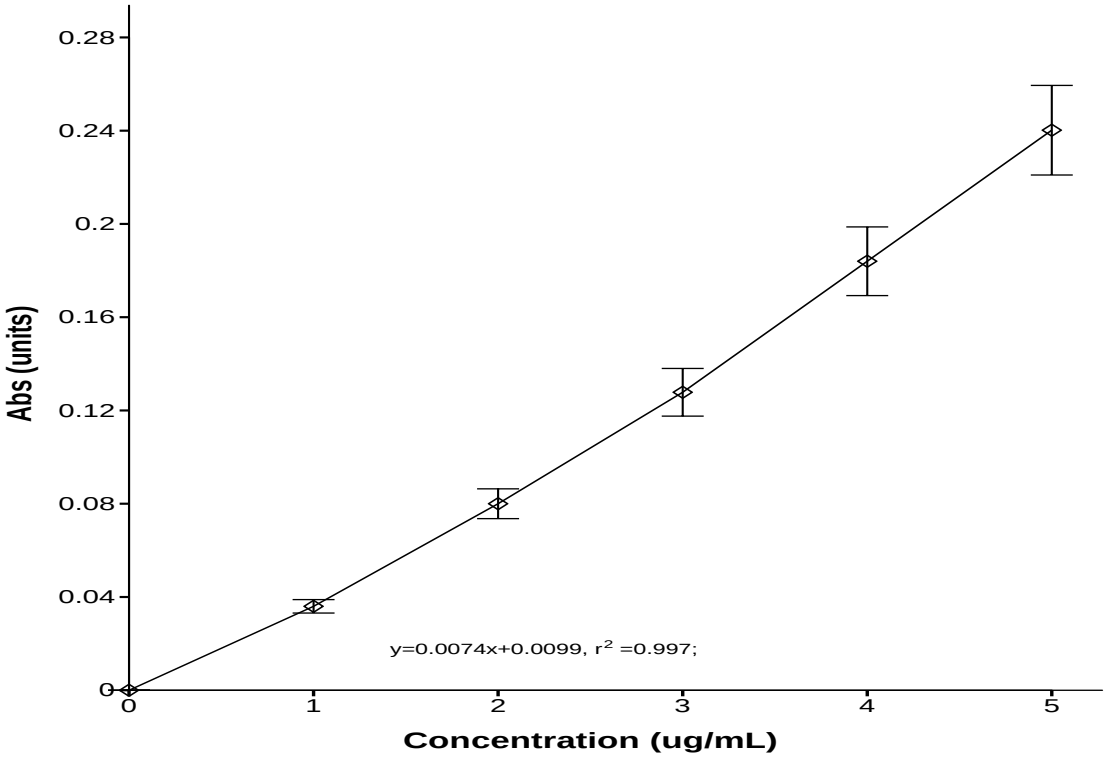

Figure 2: Standard curve of Andrographolide in methanol

FTIR spectrum of drug sample and drug sample with excipient were given in the **Figure** (**3 & 4** respectively) measured in the range of 400 to 4000 cm^{-1}. Interpretation of FTIR spectra of sample showed that the vibration frequencies observed at 3500, 1900, and 2500 cm^{-1} could be due to functional groups viz. –OH, -NH_2, and benzene ring in its chemical structure and found to be almost identical to the reference spectrum of drug. There are no significantly changes between the drug excipient spectra so drug and excipient both are compatible [39].

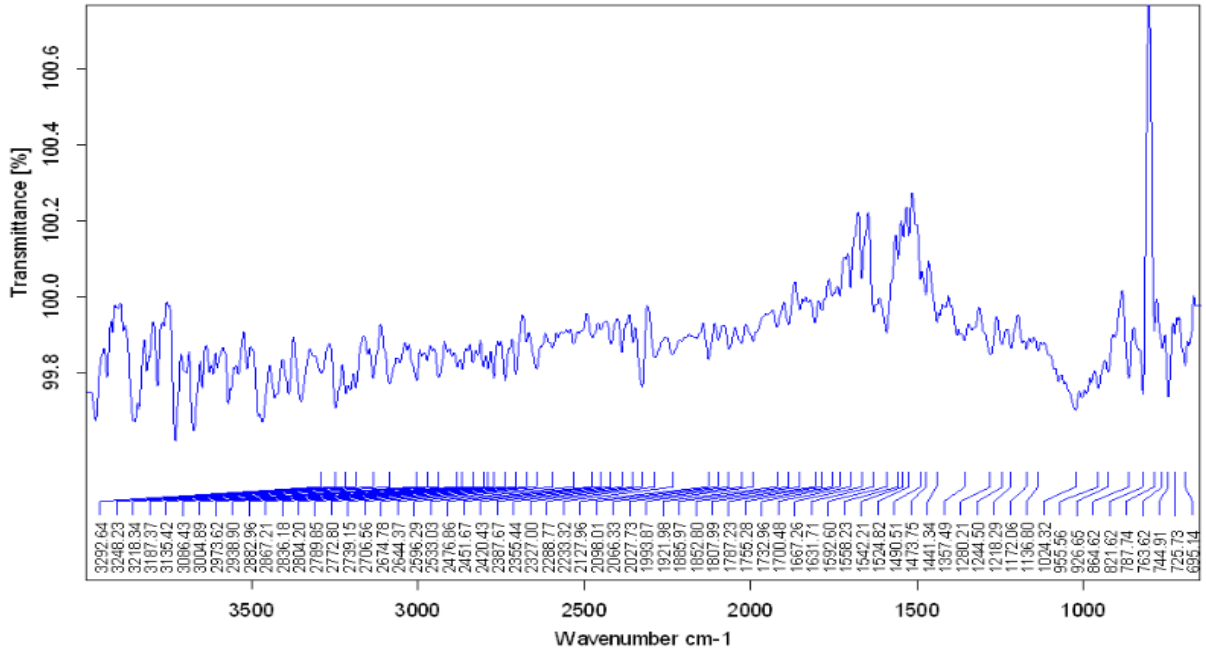

Figure 3: FTIR spectrum of procured drug sample (Andrographolide)

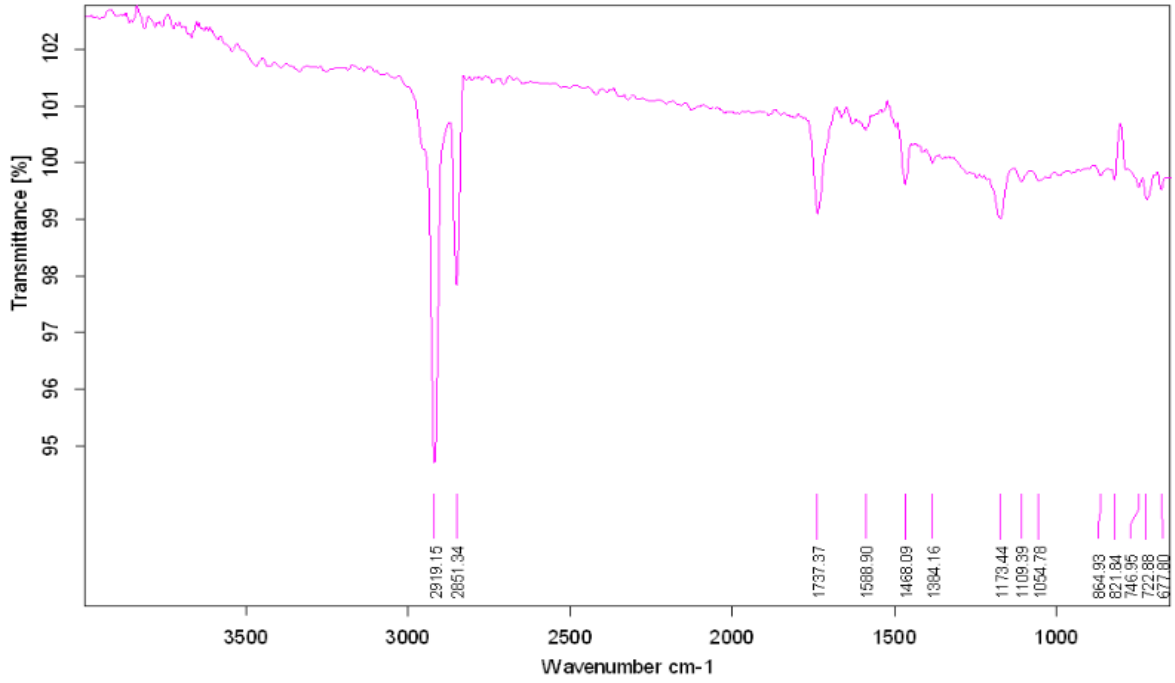

Figure 4: FTIR spectrum of drug (Andrographolide) ± Excipient mixture

Differential scanning calorimeter (DSC) characterization of sample was carried out using crippling pan method in the scanning range of 30 to 400°C. Result of DSC analysis is given in the **Figure 5 & 6** respectively**.** It indicates from the results that two thermal events were

observed; one melting endothermic appeared at 105.231°C with ΔHt value 711.527 J/g while second endothermic event observed at 316.266°C with ΔHt value 134.555 J/g revealed that drug sample possesses different polymorph or impurities with high melting range may be present [40]. Above results are confirmed by melting point determination carried out using capillary tube method, indicated its melting point at 235°C. On the basis of above results, it can be said that the procured of sample was Andrographolide.

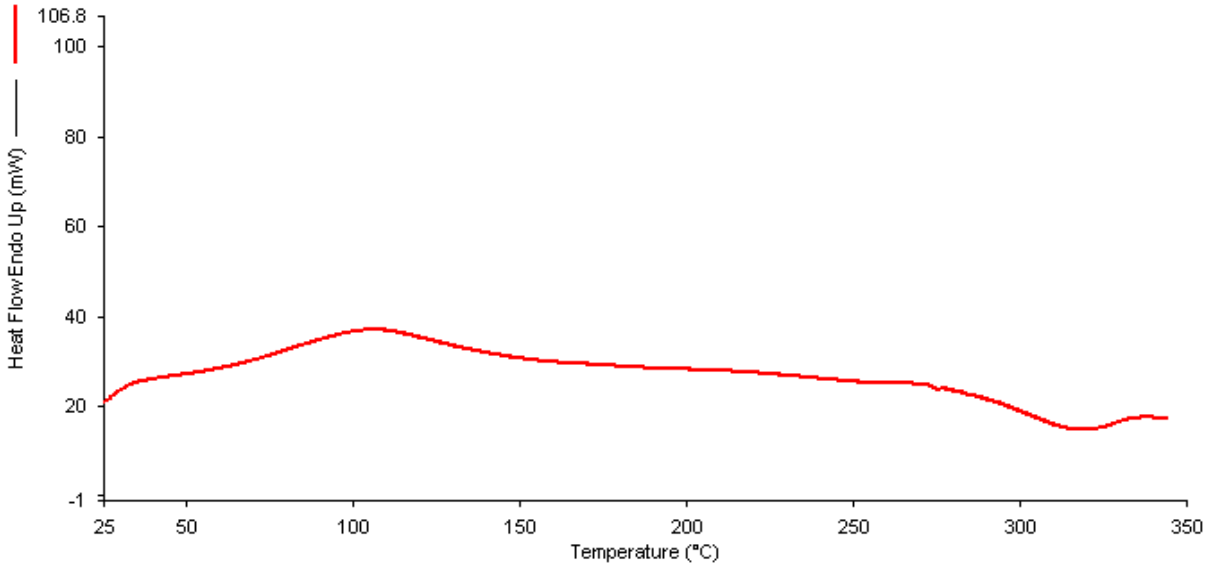

Figure 5: DSC thermo gram of Andrographolide

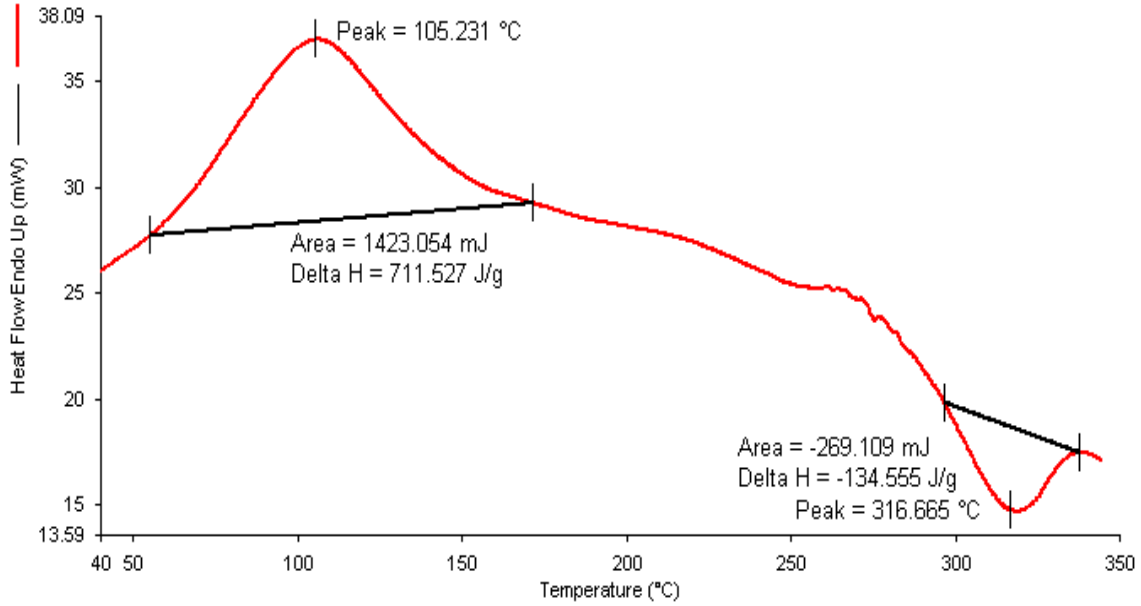

Figure 6: DSC thermo gram of Andrographolide with calculation

Particle size, polydispersity index and zeta potential

Instrument Zetasizer was utilized to ascertain the mean particle size of AND-SLNs' and polydispersity index also shown in (**Table 3**). The smallest PDI values indicate the homogenous dispersion of all the formulations and narrow particle size distribution. Actuality, the SLNs dispersion with PDI value between 0.03 and 0.06 can be classified as mono-disperse, narrowly scattered between the range 0.1 and 0.2 and broadly distributed between the range 0.25 and 0.5. Through electrostatic repulsion the zeta potential or surface charge of nanoparticles can have a significant impact on the stability of the particles in

suspension. The zeta potential of optimized formulation AND-SLNs was found to be 22.86Mv [43]. The potential difference between the dispersion medium and stationary fluid layer affixed to the dispersed particle is known as zeta potential. Particle stability in dispersion is contingent upon the equilibrium of repulsive and attraction forces between near and other particles.

Table 3 Characterization of Andrographolide-loaded nanoparticles

S No.	Formulation code	Particle size	polydispersity index	% Entrapment efficiency	Zeta Potential
1.	F1	197.56	0.09 ± 0.01	76.61	-18.98
2.	F2	238.01	0.11 ± 0.06	91.96	-12.98
3.	F3	301.65	0.09 ± 0.05	92.3	-22.86

Surface morphology

The prepared AND-SLNs formulation were characterized on the behalf of shape and surface morphology of particles, particle size, PDI value, zeta potential, % of drug entrapment efficiency, % of drug loading and *in-vitro* drug release in PBS (pH 7.4). The nanoparticles' surface morphology was examined using a transmission electron microscope. The smoother surface of AND-SLNs is demonstrated in (**Figure 7**).

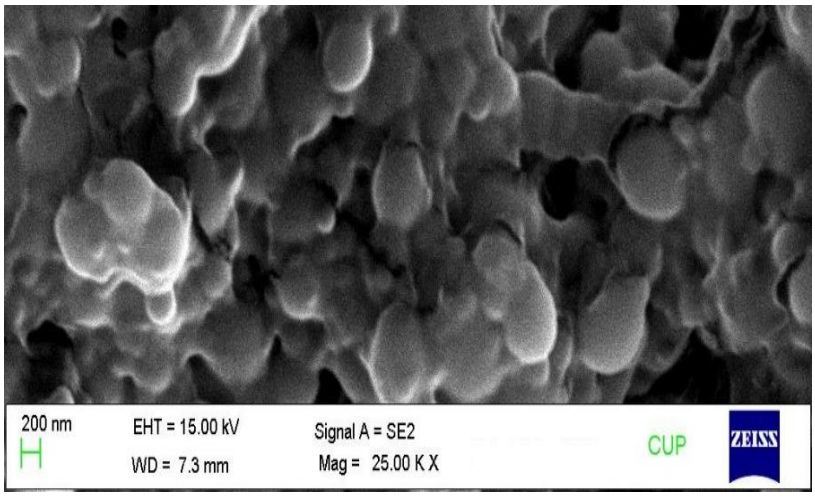

Figure 7: Transmission electron microscopy of andrographolide-loaded nanoparticles

Entrapment efficiency and in-vitro release

Using the ultracentrifugation instrument (Remi) at 20,000rpm for 30mins at 4°C, the percentage of entrapped andrographolide drug in AND-SLNs was ascertained after the nanoparticles were separated from the medium containing non-entrapped drug [44]. The supernatant was diluted with PBS (pH 7.4) before the amount of free drug was measured at 235 nm using a UV-visible spectrophotometer (Shimadzu 18 000). Additionally, the encapsulation efficiency of AND-SLNs was found to be 92.3%.

The in-vitro release profile of contained medication from AND-SLNs formulation was investigated through dialysis membrane in PBS (pH 7.4). The rate of *in-vitro* drug release from AND-SLNs formulation was found to be 59.2 (**Figure 8**). It is clear that an in-vitro drug release research from AND-SLNs formulations demonstrated an initial burst release of drug, which may have been caused by drug molecules adsorbed on the outer surface of

nanoparticles' and then showed very slow released [45]. The increases the drug release profile in case of AND-SLNs formulation as compared with pure extract formulation.

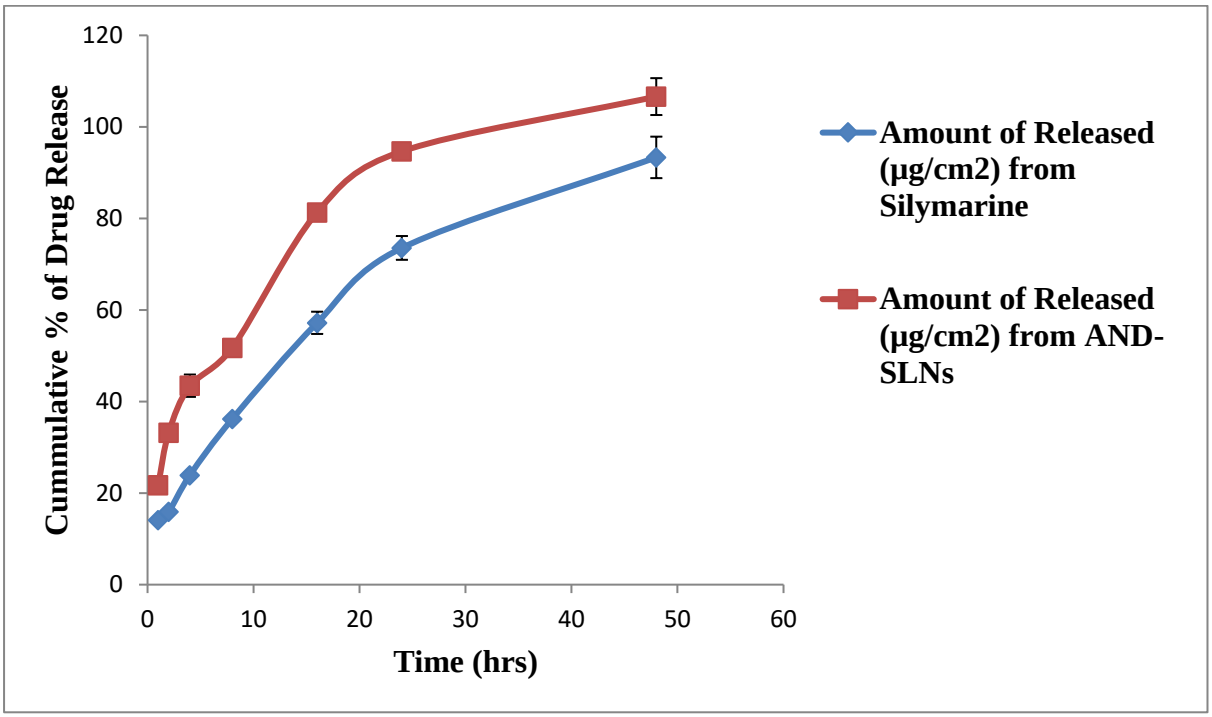

Figure 8: % Cumulative AND-SLNs formulation released in phosphate buffered saline (pH 7.4) difference between AND-SLNs and Standard drug

Conclusion

The results indicated that encapsulation of andrographolide in solid lipid nanoparticles improvement in the residence time as well as drug concentration in the targeted site like liver which could be utilized to reducing the dosing frequency as well as the dose of the formulation. Current study shows that andrographolide more potent drug against the CCl_4-induced hepatic injury through a decrease in hepatic oxidative stress.

LIST OF ABBREVIATIONS

AND-SLN: Andrographolide Nanoparticles; bw: body weight; CCl_4: Carbon tetra chloride; EE: Entrapment Efficiency; LC: Loading capacity.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE- Yes

COMPETING INTERESTS- The author has declared that no conflicts of interest exist.

FUNDING- No financial support

AUTHOR'S CONTRIBUTION- In the present Research, MK perform all the experimental procedure and analyzed the data related to nanoparticles preparation approaches and were the most important contribution in making the manuscript. SK contributed in the in-vitro drug release experimental work. NS elaborated the formulation part in the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Amudha Murugan, Krishna Kumara, Shanmugasundaram, 2014. Biosynthesis and characterization of silver nanoparticles using the aqueous extract of vitex negundo. linn. World J. Pharm. pharm. Sci., 3(8): 1385-1393
2. Kavitha, K.S., Syed Baker, Rakshith, D., Kavitha, H.U., Yashwantha Rao, H.C., Harini B., Pand Satish, S. 2013. Plants as green source towards synthesis of nanoparticles. Int. Res. J. Biol. Sci., 2 (6): 66-76.
3. Jannathul Firdhouse, M., Lalitha, P., and Shubashini, K., Sripathi, 2012. Novel synthesis of silver nanoparticles using leaf ethanol extract of Pisoniagrandis (R.Br). Der Pharma Chemica, 4 (6):2320-2326.
4. Shamay, Y., Shah, J., Isik, M., Mizrachi, A., Leibold, J., Tschaharganeh, D. F., et al. (2018). Quantitative self-assembly prediction yields targeted nanomedicines. Nat. Mater. 17, 361–368. doi: 10.1038/s41563-017-0007-z
5. Wei, L., Lu, J., Xu, H., Patel, A., Chen, Z. S., and Chen, G. (2015). Silver nanoparticles: synthesis, properties, and therapeutic applications. Drug Discov. Today 20, 595–601. doi: 10.1016/j.drudis.2014.11.014
6. Zeng, L., Gupta, P., Chen, Y., Wang, E., Ji, L., Chao, H., et al. (2017). The development of anticancer ruthenium (ii) complexes: from single molecule compounds to nanomaterials. Chem. Soc. Rev. 46, 5771–5804. doi: 10.1039/ c7cs00195a
7. Arms, L., Smith, D. W., Flynn, J., Palmer, W., Martin, A., Woldu, A., et al. (2018). Advantages and limitations of current techniques for analyzing the biodistribution of nanoparticles. Front. Pharmacol. 9:802. doi: 10.3389/fphar. 2018.00802
8. Maeki, M., Kimura, N., Sato, Y., Harashima, H., and Tokeshi, M. (2018). Advances in microfluidics for lipid nanoparticles and extracellular vesicles and applications in drug delivery systems. Adv. Drug Deliv. Rev. 128, 84–100. doi: 10.1016/j.addr.2018.03.008
9. Kohay, H., Sarisozen, C., Sawant, R., Jhaveri, A., Torchilin, V. P., and Mishael, Y. G. (2017). PEG-PE/clay composite carriers for doxorubicin: effect of composite structure on release, cell interaction and cytotoxicity. Acta Biomater. 55, 443–454. doi: 10.1016/j.actbio.2017.04.008
10. Wu, J., Yuan, J., Ye, B., Wu, Y., Xu, Z., Chen, J., et al. (2018). Dual-responsive core cross linking glycopolymer-drug conjugates nanoparticles for precise hepatocarcinoma therapy. Front. Pharmacol. 9:663. doi: 10.3389/fphar.2018. 00663
11. R. A. Kumar, K. Sridevi, N. Vijaya Kumar, S. Nanduri, and S. Rajagopal, “Anticancer and immune stimulatory compounds from Andrographis paniculata,” Journal of Ethno pharmacology, vol. 92, no. 2-3, pp. 291–295, 2004.
12. S. Rajagopal, R. A. Kumar, D. S. Deevi, C. Satyanarayana, and R. Rajagopalan, “Andrographolide, a potential cancer therapeutic agent isolated from Andrographis

paniculata," Journal of Experimental Therapeutics and Oncology, vol. 3, no. 3, pp. 147–158, 2003.

13. C. Calabrese, S. H. Berman, J. G. Babish et al., "A phase I trial of andrographolide in HIV positive patients and normal volunteers," Phytotheraphy Research, vol. 14, no. 5, pp. 333–338, 2000.
14. P. K. Singha, S. Roy, and S. Dey, "Antimicrobial activity of Andrographis paniculata," Fitoterapia, vol. 74, no. 7-8, pp. 692 – 694, 2003.
15. K. Jarukamjorn and N. Nemoto, "Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent andrographolide," Journal of Health Science, vol. 54, no. 4, pp. 370–381, 2008.
16. T. Fujita, R. Fujitani, Y. Takeda et al., "On the ditrpenoids of Andrographis paniculata: X-ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids," Chemical & Pharmaceutical Bulletin, vol. 32, no. 6, pp. 2117–2125, 1984.
17. C. J. Medforth, R. S. Chang, G. Q. Chen, M. M. Olmstead, and K. M. Smith, "A conformational study of diterpenoid lactones isolated from the Chinese medicinal herb Andrographis paniculata," Journal of the Chemical Society, Perkin Transactions 2, no. 6, pp. 1011–1016, 1990.
18. M. Rajani, N. Shrivastava, and M. N. Ravishankara, "A rapid method for isolation of andrographolide from Andrographis paniculata Nees (Kalmegh)," Pharmaceutical Biology, vol. 38, no. 3, pp. 204–209, 2000.
19. Q. Du, G. Jerz, and P. Winterhalter, "Separation of andrographolide and neoandrographolide from the leaves of Andrographis paniculata using high-speed counter-current chromatography," Journal of Chromatography A, vol. 984, no. 1, pp. 147–151, 2003.
20. L. Cui, F. Qiu, and X. Yao, "Isolation and identification of seven glucuronide conjugates of andrographolide in human urine," Drug Metabolism and Disposition, vol. 33, no. 4, pp. 555–562, 2005.
21. L. Lomlim, N. Jirayupong, and A. Plubrukarn, "Heataccelerated degradation of solid-state andrographolide," Chemical & Pharmaceutical Bulletin, vol. 51, no. 1, pp. 24–26, 2003.
22. L. H. Qin, L. Kong, G. J. Shi, Z. T. Wang, and B. X. Ge, "Andrographolide inhibits the production of TNF-α and interleukin-12 in lipopolysaccharide-stimulated macrophages: role of mitogen-activated protein kinases," Biological & Pharmaceutical Bulletin, vol. 29, no. 2, pp. 220–224, 2006.
23. Poolsup N, Suthisisang C, Prathanturarug S, Asawamekin A, Chanchareon U. Andrographis paniculata in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials. J Clin Pharm Ther 2004; 29(1): 37-45.
24. K. Maiti, A. Gantait, K. Mukherjee, B. P. Saha, and P. K. Mukherjee, "Therapeutic potentials of andrographolide from Andrographis paniculata: a review," Journal of Natural Remedies, vol. 6, no. 1, pp. 1–13, 2006.
25. V. J. Ram, "Herbal preparations as a source of hepatoprotective agents," Drug News and Perspectives, vol. 14, no. 6, pp. 353–363, 2001.

26. J. S. Rajkumar,M. G. Sekar, and S. K. Mitra, "Safety and efficacy of oral HD-03/ES given for six months in patients with chronic hepatitis B virus infection," World Journal of Gastroenterology, vol. 13, no. 30, pp. 4103–4107, 2007.
27. B. Shukla, P. K. S. Visen, G. K. Patnaik, and B. N. Dhawan, "Choleretic effect of andrographolide in rats and guinea pigs," Planta Medica, vol. 58, no. 2, pp. 146–149, 1992.
28. A. C. Rana and Y. Avadhoot, "Hepatoprotective effects of Andrograhphis paniculata against carbon tetra chloride induced liver damage," Archives of Pharmacal Research, vol. 14, no. 1, pp. 93–95, 1991.
29. P. K. S. Visen, B. Shukia, G. K. Patnaik, and B. N. Dhawan, "Andrographolide protects rat hepatocytes against paracetamol-induced damage," Journal of Ethno pharmacology, vol. 40, no. 2, pp. 131–136, 1993.
30. Karakus E, Karadeniz A, Simsek N, Can I, Kara A, Yildirim S, et al. Protective effect of Panax ginseng against serum biochemical changes and apoptosis in liver of rats treated with carbon tetrachloride (CCl4). J Hazard Mater 2011; 195:208–13.
31. Cemek M, Aymelek F, Buyukokuroglu ME, Karaca T, Buyukben A, Yilmaz F. Protective potential of Royal Jelly against carbon tetrachloride induced-toxicity and changes in the serum sialic acid levels. Food Chem Toxicol 2010;48:2827–32.
32. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al. Acetaminophen induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 2005; 42: 1364–72.
33. Domenicali M, Caraceni P, Giannone F, Baldassarre M, Lucchetti G, Quarta C, et al. A novel model of CCl4-induced cirrhosis with ascites in the mouse. J Hepatol 2009; 51: 991–9.
34. Lee CH, Park SW, Kim YS, Kang SS, Kim JA, Lee SH, et al. Protective mechanism of glycyrrhizin on acute liver injury induced by carbon tetrachloride in mice. Biol Pharm Bull 2007; 30:1898–904.
35. Yang X, Yang S, Guo Y, Jiao Y, Zhao Y. Compositional characterisation of soluble apple polysaccharides, and their antioxidant and hepatoprotective effects on acute CCl4- caused liver damage in mice. Food Chem 2013; 138:1256–64.
36. Rechnagel RO, Glende Jr EA. Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. CRC Crit Rev Toxicol 1973; 2:263–97.
37. Hung MY, Fu TY, Shih PH, Lee CP, Yen GC. Du-Zhong (Eucommia ulmoides Oliv) leaves inhibits CCl4-induced hepatic damage in rats. Food Chem Toxicol 2006;44: 1424–31.
38. Shen X, Tang Y, Yang R, Yu L, Fang T, Duan JA. The protective effect of Zizyphus jujube fruit on carbon tetrachloride-induced hepatic injury in mice by anti-oxidative activities. J Ethnopharmacol 2009; 122:555–60.
39. Tugarova, A. V., Mamchenkova, P. V., Dyatlova, Y. A., & Kamnev, A. A. (2018). FTIR and Raman spectroscopic studies of selenium nanoparticles synthesised by the bacterium Azospirillum thiophilum. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 192, 458–463.

40. Durowoju, I. B., Bhandal, K. S., Hu, J., Carpick, B., & Kirkitadze, M. (2017). Differential Scanning Calorimetry — A Method for Assessing the Thermal Stability and Conformation of Protein Antigen. Journal of Visualized Experiments, (121).
41. Hassan, S. S. M. (1980). Colorimetric determination of vitamin D2 (Calciferol). Methods in Enzymology, 323–326.
42. Luykx, D. M. A. M., Peters, R. J. B., van Ruth, S. M., & Bouwmeester, H. (2008). A Review of Analytical Methods for the Identification and Characterization of Nano Delivery Systems in Food. Journal of Agricultural and Food Chemistry, 56(18), 8231–8247.
43. Kawakami S *et al*. Asialoglycoprotein receptor-mediated gene transfer using novel galactosylated cationic liposomes. *Biochem Biophys Res Commun* 1998; 252: 78–83.
44. Mishra D.,et al. Glycyrrhizin conjugated chitosan nanoparticles for hepatocyte-targeted delivery of lamivudine. Journal of Pharmacy and Pharmacology. 2014; 1-12.
45. Mandloi DK *et al*. Method development and validation of RP-HPLC in the application of in vitro dissolution study of lamivudine in bulk drug and tablet formulation. *J Chem Pharm Res* 2009; 1: 286–296.
46. Calvo P *et al*. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carrier. *J Appl Poly Sci* 1997; 63: 125–132.