https://doi.org/10.33472/AFJBS.6.Si2.2024.3111-3130

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Hepatoprotective Effect of Glycyrrhetinic Acid mediated Andrographolide Solid Lipid Nanoparticles formulation from *Andrographis Paniculata,* against Carbon Tetrachloride (CCl_4) Induced Hepatotoxicity

Mukesh Kumar[1], Sokindra Kumar[1], Nitin Sharma[2]

[1] Faculty of Pharmacy, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subhati University, Subhartipuram NH-58, Delhi-Haridwar Bypass Road, Meerut, Uttar Pradesh 250005 India

[2] Dr. Nitin Sharma, Department of Pharmaceutics, Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, 201301, India

Corresponding Author*:Prof. (Dr.) Sokindra Kumar

Volume 6, Issue Si2, 2024

Received: 13 March 2024

Accepted: 14 April 2024

Published: 20 May 2024

doi:10.33472/AFJBS.6.Si2.2024.3111-3130

ABSTRACT

Liver is a largest vital organ used for the detoxification of toxic material present in the body because excessive toxic material causes hepatic injury. This study was focused on preparation of G-AND-SLNs using solvent evaporation method for liver targeting. The prepared G-AND-SLNs were characterized using transmission electron microscopy. The drug-loaded G-AND-SLNs encapsulation efficiency and behaviour of *in-vitro* drug release were studied using UV spectroscopy. The release of active moiety andrographolide from the solid lipid nanoparticles showed a biphasic pattern, burst release initial pattern and secondly maintain sustained release. Haematological parameter study shows decline of the tissue damage or tissue mortality in comparison with plain pharmaceutical solution. Under the present study was evaluate the possible hepatoprotective effects of andrographolide against the carbon tetrachloride (CCl_4) induced hepatic injury in rats. Animals (Rats) were divided into five groups. Hepatotoxicity was induced by the administration of a single oral dose of CCl_4 in experimental rats. 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. The administration of CCl_4 resulted in marked alteration in serum hepatic enzymes (like AST, ALT and ALP), oxidant parameters (like GSH and MDA) indicative of hepatic injury. Treatment with standard drug, silymarin also reversed CCl_4 induced changes in biomarkers of liver function, oxidant parameters and inflammation. The biochemical observations were paralleled by histopathological findings in rat liver both in the case of CCl_4 and treatment groups. In conclusion, andrographolide produced a protective effect against CCl_4-induced liver damage. Our study suggests that Andrographolide may be used as a hepatoprotective agent against toxic effects caused by CCl_4 and other chemical agents in the liver.

Key words: Andrographolide, Liver Targeting, Anti-oxidant, Carbon tetrachloride (CCl_4), Hepatoprotection and Biodistribution.

Introduction

Liver is a largest and critical organ that plays an important role in the conjugation and detoxification of numerous medications **(Karakus E. et al. 2011)**. 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 **(Cemek M. et al. 2010)**. Drug paracetamol **(Larson A.M. et al. 2005)**, carbon tetrachloride (CCl_4) **(Domenicali M. et al. 2009)**, 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 **(Lee C.H. et al. 2007)**. 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 **(Yang X. et al. 2013)**. 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 **(Rechnagel R.O. et al. 1973)**. CCl_4 produces liver damage through many routes **(Shen X. et al. 2009)**. CCl_4 is considered to cause hepatotoxicity by enhanced lipid peroxidation caused by free radical production **(Shen X. et al. 2009)**. 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.

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 **(Kumar R.A. et al. 2004)**. The plant's extracts and andrographolide shows various Pharmacological properties include immunostimulatory **(Rajagopal S. et al. 2003)**, antiviral **(Calabrese C. et al. 2000)**, and antibacterial actions **(Singh P.K. et al. 2003)**. Primary effective chemical ingredient, andrographolide has shows a wide or broad spectrum biological actions, including various effects **(Jarukamjorn K. et al. 2008)**. A. paniculata contains andrographolide, a significant bioactive phytoconstituent, in a variety of tissues, but especially leaves **(Fujita T. et al.**

1984). 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%) **(Medforth C.J. et al. 1990** & **Rajani M. et al. 2000)**. These are the numerous methods that have been utilized to investigate the structure of **(Du Q. et al. 2003** & **Cui L. et al. 2005)**. 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 **(Lomlim L. et al. 2003)**. 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 **(Qin L.H. et al. 2006)**. 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 **(Poolsup N. et al. 2004)**. Moreover, andrographolide commonly used in various polyherbal hepatoprotective formulations **(Maiti K. et al. 2006)**. It has been shown to be better effective in hepatitis B treatment **(Ram V.J. et al. 2001)**. According to literature review, andrographolide was reduces the liver problems associated with various drugs and chemicals **(Rajkumar J.S. et al. 2007)**. 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 **(Shukla B. et al. 1992)**. 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) **(Rana A.C. et al. 1991)**. A. paniculata extract demonstrated hepatoprotective properties in line with its traditional uses and pharmacology **(Visen P.K.S. et al. 1993)**.

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 **(Murugan A. et al. 2014)**. Since nanoparticles (NP) offer sustained-release action and therapeutic targeting, drug delivery to hepatocytes via NP is particularly interesting **(Kavitha K.S. et al. 2013)**. One method for selective medication delivery that shows promise is receptor-mediated drug targeting **(Firdhouse J. et al. 2012)**. To improve

selectivity toward liver cells, an effective strategy is to design nano-sized carrier to realize liver-targeted delivery **(Shamay Y. et al. 2018)**. Recently, nanoparticles have been proved to have the advantages in drug delivery with low system toxicity **(Wei L. et al. 2015** & **Zeng L. et al. 2017)**. Many nano-sized drug delivery systems **(Arms L. et al. 2018)**, such as natural and synthetic polymer nanoparticles, metal nanoparticles, and polymer-drug conjugates, have been investigated for delivery of hepatostimulant and hepatoprotective drugs **(Maeki M. et al. 2018)**. 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 **(Kohay H. et al. 2017)**. In the past decade, many efforts have been made to prepare liver-targeting nano-carriers **(Wu J. et al. 2018)**.

Material and Method

Drug and chemical

The drug andrographolide was purchased from Ambe NS Agro Product (with a purity ≥ 98%). Stearic acid, Pluronic and silymarin 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.

Experimental animal

After animal ethical approval no. 1204/PO/Re/S/08/CPCSEA/23-03 in this study male albino wistar 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 of preparation of SLNs

The glycyrrhetinic acid mediated andrographolide loaded SLNs were prepared by using the solvent evaporation method. Briefly, the organic phase was prepared by dissolving andrographolide (100 mg) and steric acid (100 to 200 mg) in 10 mL of methanol. And the aqueous phase was prepared by dissolving surfactant (Pluronic-127) and co-surfactant (75 to 150 mg) emulsion was prepared by rapidly dispersed the organic phase drop by drop in to the 20 mL aqueous phaseunder probe sonication for 8.5 minutes. Finaly the emulsion was stirred at 10000 rpm for 2 h. Finally, the temperature of the system was elevated at temperature 75 C. The developed glycyrrhetinic acid mediated andrographolide solid lipid nanoparticles were separated by freeze-drying.

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 **(Kawakami S. et al. 1998)**.

Surface morphology

The morphology of solid lipid nanoparticles was observed using scanning electron microscopy (JEM-2010 HR, JEOL, and Japan). On a copper grid, a single drop of the nanoparticle suspension was stained for two minutes using a 2% phosphotungstic acid solution **(Mahajan H. et al. 2017)**. 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 Corp A116353) was used to measure the amount of free andrographolide in the supernatant at a wavelength of 225 nm **(Mishra D. et al. 2014)**. 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 10 mg 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 225nm **(Mandloi D.K. et al. 2009)**.

Hematological parameters liver tissue

Rats were randomly distributed in to five groups and were given oral dosing: Group-I (n=6): control; Group-II (n=6): treated with carbon tetra chloride; Group-III (n=6): CCl_4 ± low dose of formulation; Group-IV (n=6): CCl_4 ± high dose of formulation; and Group-V (n=6): CCl_4 ± Standard drug. Hepatic toxicity in animals was developed using carbon tetra chloride model (CCl_4): After 24 hrs, the blood samples were collected from retro orbital plexus using capillary tube method. The following hematological parameters were determined **(Gupta S. et al. 2017)**.

Biochemical Estimation

Rats were decapitated under the anesthesia for 24hrs after hepatotoxicity. The liver tissue was removed and rinse with saline. The impaired liver tissue was dissected and separated out. For the evaluation of biochemical parameter like malondialdehyde (MDA), reduced glutathione (GSH). Liver tissue was homogenized using 0.1M phosphate buffer (pH 7.4). Protein concentration was determined **(Gupta S. et al. 2017)**.

Procedure for the preparation of homogenate

Take 30mg liver tissue of each sample was weighed and added 300µl lysis buffer. Tissue with lysis buffer was homogenized with the help of homogenizer or motor pastel. All the sample and usable kept in freeze dried condition. Homogenate was kept in ice for 15min. homogenate was centrifuged for 15min at 1500 rpm at 4°C **(Placer Z.A. et al. 1966)**.

Determination of oxidative stress markers

Determination of lipid peroxides, measured as malondialdehyde (MDA)

The lipid peroxidation indicator MDA was calculated. In 100µl in proceed liver tissue homogenate sample, 100µl trichloro acetic acid (TCA) and 100µl of thiobarbituric acid (TBA) were added and gently vortexed the sample. The mixture was than heated at 90°C for 15min in a water bath. The above mixture was cooled using tap water and pink color is appeared. Above cooled mixture is centrifuged at 300rpm for 15min. Sample was poured into wells (ELISA Plate) and read sample at 535nm. The MDA concentration is expressed in the term of nM/mg of protein **(Ohkawa H. et al. 1979)**.

Determination of Reduced Glutathione Levels (GSH)

The GHS levels were calculated using suitable methods. Take 50µl of liver tissue homogenate sample, 150µl tris buffer, 10µl DTNB and 790µl of methanol were added. The sample mixture kept in dark place for 15min at room temperature. After 15min centrifuged the sample mixture at 2000rpm for 15min. sample was poured into wells (ELISA Plate) and read the sample at 412nm. The GSH concentration is expressed in the term of µg/mg of protein **(Ellman G.L. et al 1959)**.

Histopathology of liver tissue

Experimental specimen was killed using cervical decapitation and the removed liver tissue was dipped fixed in 10% formalin solution immediately. Histopathological studies on liver tissues of rats were observed using haematoxylin, followed by staining of eosin, under a high resolution microscopic observation **(Pangeni R. et al. 2014)**.

Biodistribution studies

Biodistribution study was carried out to analyze the distribution pattern of G-AND-SLNs in various organs of the body. Male rat's animals weighing 200 ± 10 g were selected for evaluating localization of the labelled complex. 99mTc-G-AND-SLN formulation was

administered through the oral route of each rat. Groups of 3 rats per time point were used in the study. The organ distribution studies of labeled G-AND-SLNs were evaluated after 15 min, 30 min, 45min, 60 min, 120 min and 240 min of post administration. At these time intervals, blood was collected by tail and the animals were humanely sacrificed. Subsequently, tissues (liver, lung, spleen and kidney) were removed, washed with normal saline to make them free from adhering tissues and then weighed. The radioactivity in each organ was counted in gamma counter **(Mishra P. et al. 1991)**.

Stability studies of optimized formulations

The stability studies of glycyrrhetinic acid mediated andrographolide SLNs were continued performed for periods of three months using stability chamber make (Mac, model CAT No MSW-127). The optimized formulation was accurately measure and filled in the amber color bottle wrapped in black paper and stored in the zipper bag and put in to the humidity chamber **(Kim K.S. et al. 2017)**. The temperature and humidity condition of the chamber was throughout maintained at 25 ± 1 °C, 25 ± 1 °C, and 40 ± 1°C. Each SLNs formulation was stored for the periods of three months. The formulations were analyzed at the end of 0, 1, 2, and 3 months **(Lei Y. et al. 2012)**. The sample formulation was analyzed for physical appearance and viscosity of the SLNs formulation.

Result and Discussion

Preparation of G-AND-SLNs

The glycyrrhetinic acid mediated andrographolide loaded SLNs were prepared by using the solvent evaporation method. Briefly, the organic phase was prepared by dissolving andrographolide (100 mg) and steric acid (100 to 200 mg) in 10 mL of methanol. And the aqueous phase was prepared by dissolving surfactant (Pluronic-127) and co-surfactant (75 to 150 mg) emulsion was prepared by rapidly dispersed the organic phase drop by drop in to the 20 mL aqueous phaseunder probe sonication for 8.5 minutes. Finaly the emulsion was stirred at 10000 rpm for 2 h. Finally, the temperature of the system was elevated at temperature 75 °C. The developed glycyrrhetinic acid mediated andrographolide solid lipid nanoparticles were separated by freeze-drying. The prepared solid lipid nanoparticles formulation concentration was shown in **(Table 1)**.

Table 1: Preparation of SLNs by using different ratio of lipid and surfactant concentration

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

Characterization of G-AND-SLNs formulations

Particle size, polydispersity index and zeta potential

All three parameter like mean particle size of G-AND-SLNs' and polydispersity index data value were shown in **(Table 2**). The zeta potential of optimized G-AND-SLNs formulation was found to be 22.86Mv **(Kawakami S. et al. 1998)**. The mean particle size and polydispersity index of the selected formulation was found to be 301.65 nm and 0.09 ± 0.05 respectively.

Table 2: 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 nanoparticles' surface morphology was examined using a Scanning electron microscope **(Mahajan H. et al. 2017)**. The smoother surface of G-AND-SLNs is demonstrated in (**Figure 1**).

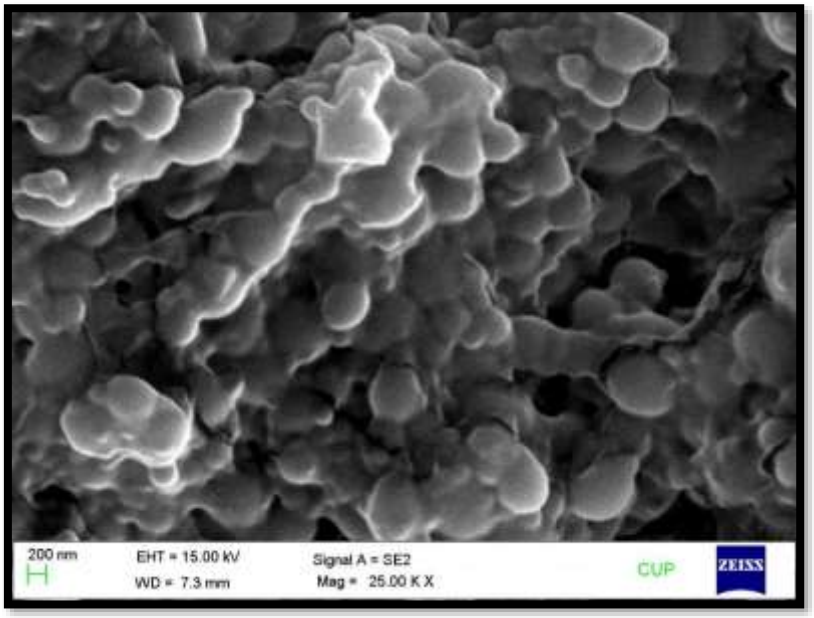

Figure 1: Scanning electron microscopy of andrographolide-loaded nanoparticles

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 G-AND-SLNs was ascertained after the nanoparticles were separated from the medium containing non-entrapped drug. The supernatant was diluted with PBS (pH 7.4) before the amount of free drug was measured at 225 nm using a UV-visible spectrophotometer (Shimadzu Corp A116353). Additionally, the encapsulation efficiency of G-AND-SLNs was found to be 92.3% **(Mishra D. et al. 2014)**.

The *in-vitro* release profile of contained medication from G-AND-SLNs formulation was investigated through dialysis membrane in PBS (pH 7.4). The rate of *in-vitro* drug release from G-AND-SLNs formulation was shown in **(Figure 2**). It is clear that an in-vitro drug release research from G-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 **(Mandloi D.K. et al. 2009)**. The increases the drug release profile in case of G-AND-SLNs formulation as compared with G-AND suspension formulation.

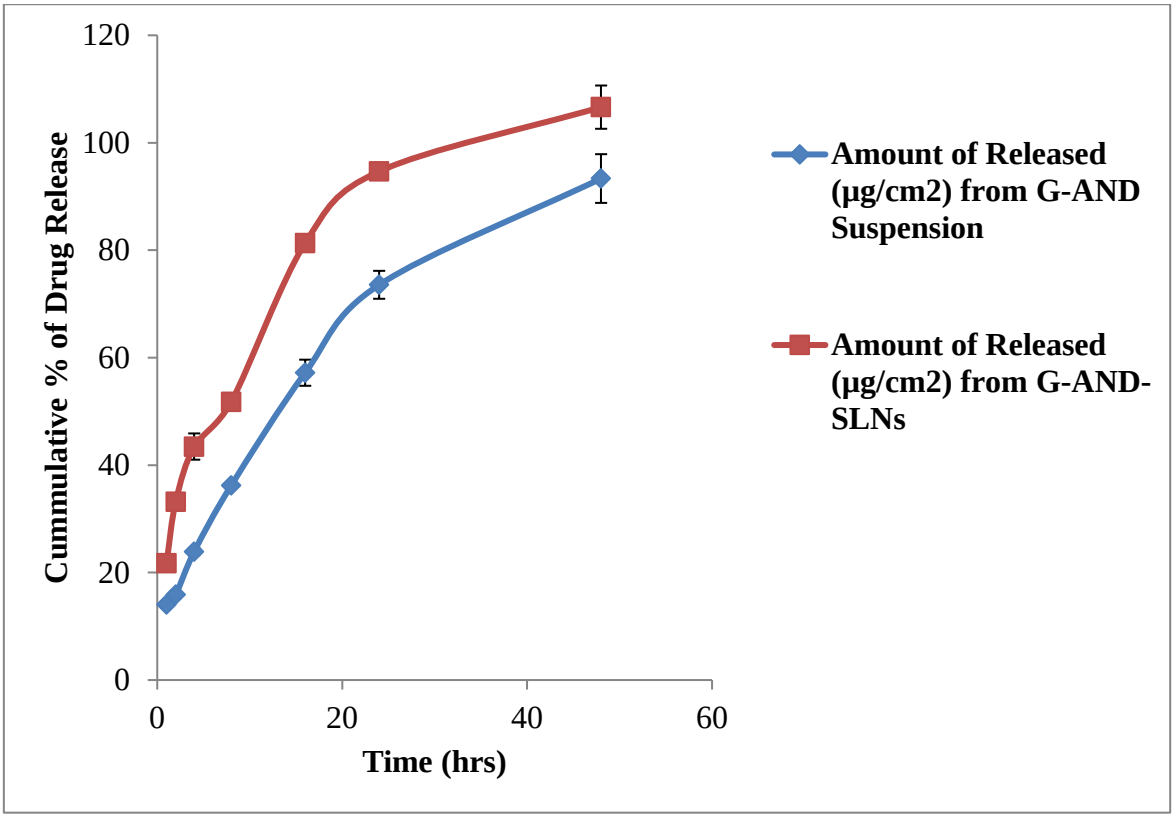

Figure 2: % Cumulative In-vitro drug permeation across rat GI mucosa from G-AND suspension & G-AND-SLNs formulation

Effects of andrographolide on CCl4-induced changes on liver function parameters in serum

The liver biomarker like AST, ALT and ALP were estimated in serum samples. In this research, administration single dose of CCl_4 to rats resulted in liver injury in rats increase the level of liver biomarker in serum in comparison to control group **(Gupta S. et al. 2017)**. Elevated liver biomarker in serum suggest increased damage to the hepatic cells by CCl_4. Andrographolide treatment significantly reversed the liver biomarker level like AST, ALT and ALP in CCl_4 induced hepatic toxicity **(Figure 3)**. Silymarin treatment also reversed CCl_4 induced changes in biomarkers of liver function. In this study the drug Silymarin used as a standard drug

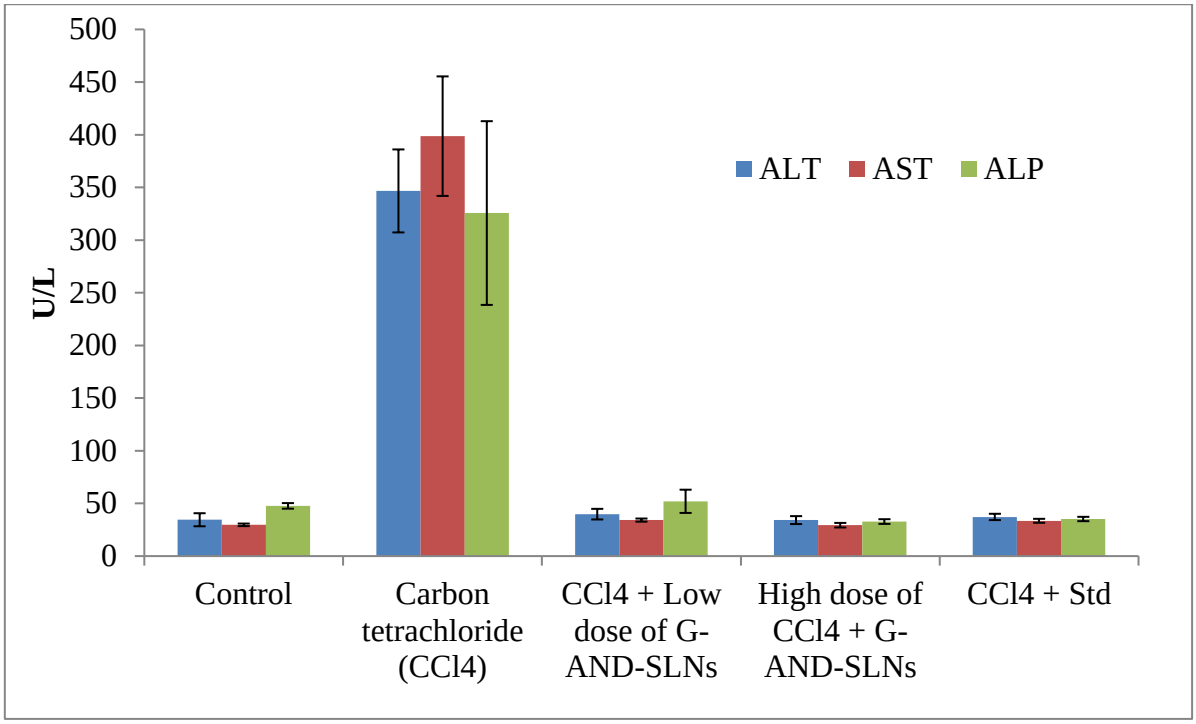

Figure 3: Effects of andrographolide on CCl4-induced changes on liver function parameters in serum of different experimental groups. The data are expressed as mean ± SEM (n=6).

Effects of riboflavin on CCl4-induced changes on parameters of oxidative stress in liver

When CCl_4 was administered, the amount of MDA in the liver was much higher than in the control group. Following andrographolide treatment **(Ohkawa H. et al. 1979)**, there was a substantial reversal of the liver MDA levels' CCl_4-induced rise **(Figure 4)**. As a result, rats given CCl_4 showed a markedly lower liver GSH level than the control group; this drop was restored by administering andrographolide **(Figure 4)**. Similar effects to andrographolide were generated by silymarin **(Ellman G.L. et al 1959)**.

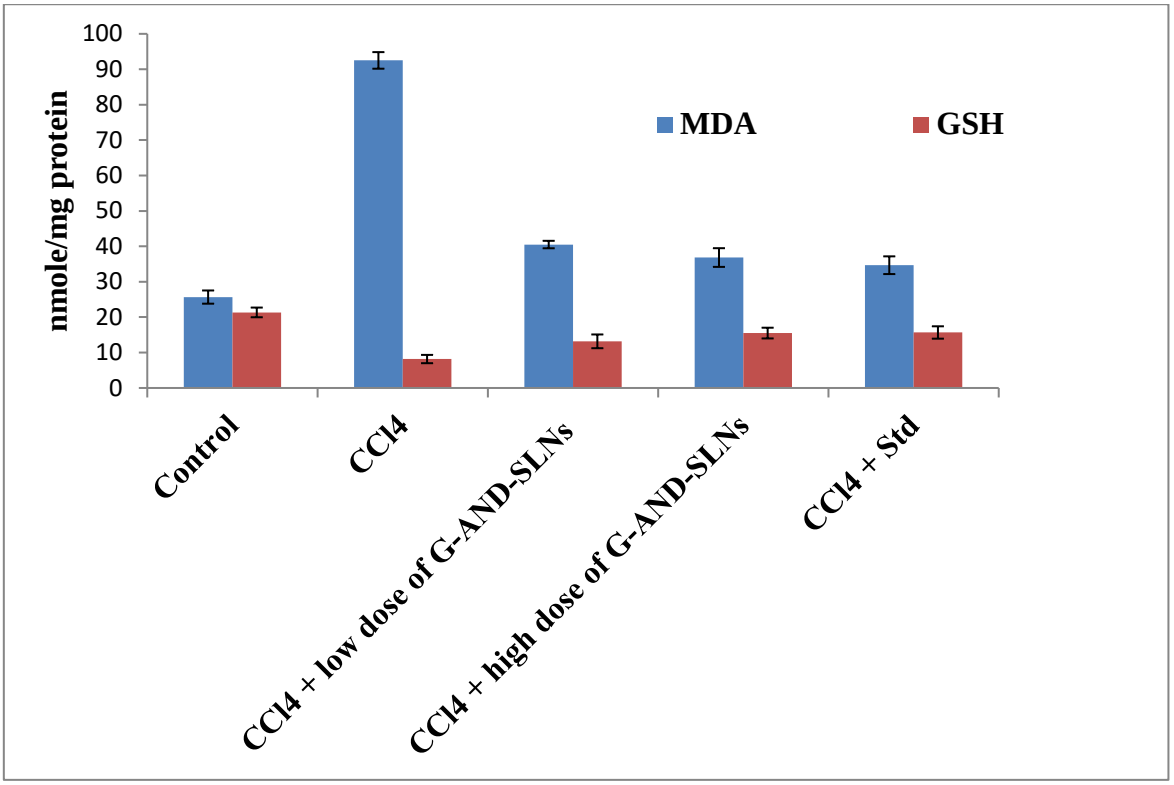

Figure 4: Effects of andrographolide on CCl_4-induced changes on parameters of oxidative stress in the liver of different experimental groups. The data are expressed as mean ± SD (n = 6).

Histology

Histological studies of various control and treated groups were performed and assess whether the G-AND-SLNs treatment would result significant retardation of liver tissue damage in comparison with plain drug suspension and histopathology result shows in **(Figure 5) (Pangeni R. et al. 2014)**.

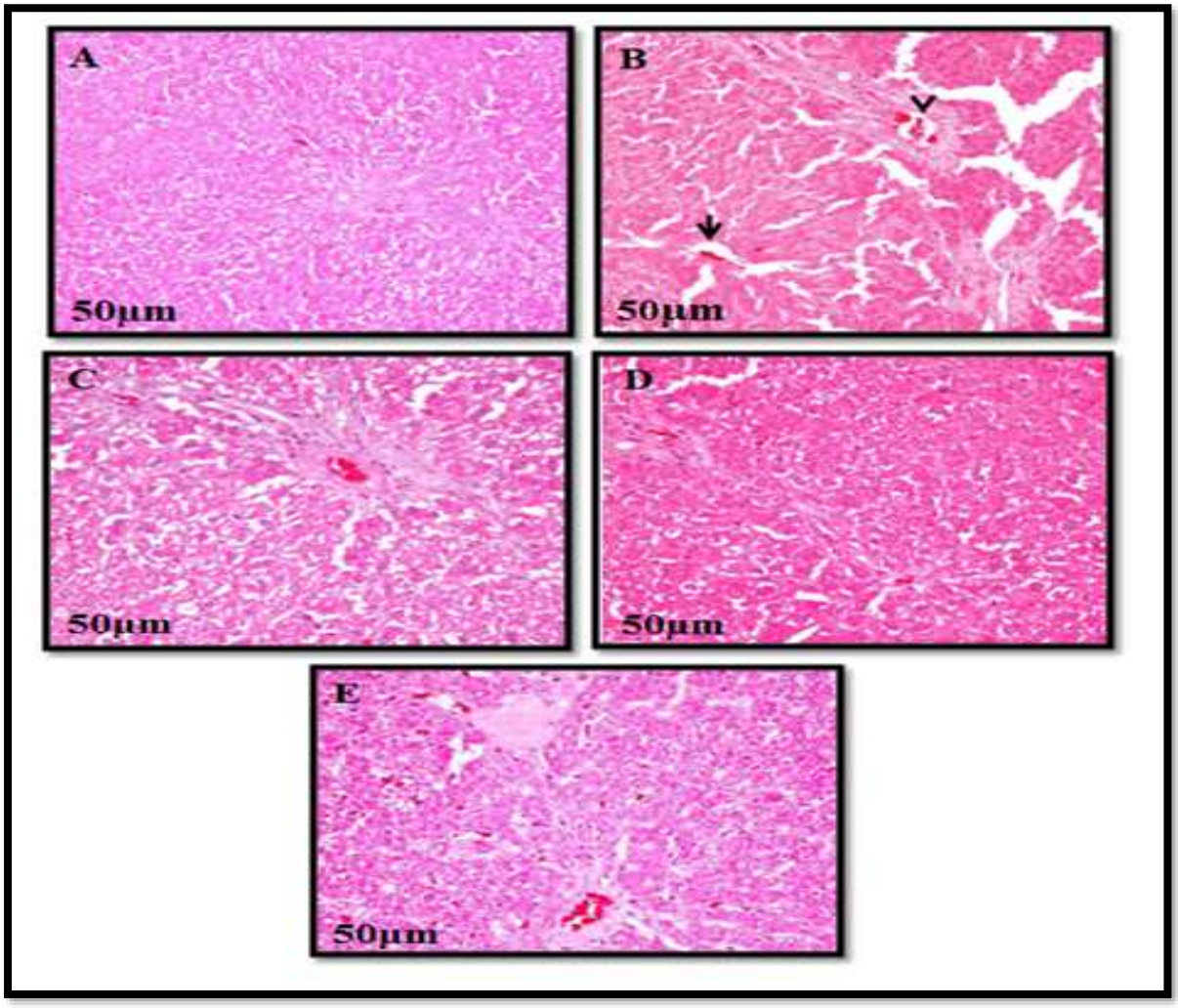

Figure 5: Effects of andrographolide on CCl_4-induced changes in liver histopathology of different experimental groups. (A) Control, (B) CCl_4, (C) low dose of formulation + CCl_4, (D) high dose of formulation + CCl_4, and (E) Silymarin + CCl_4. (n=6 animal per group; magnification=10×40x). Arrow head and double arrow heads indicate hepatocyte ballooning, necrosis and inflammation of central vein, and pericellular fibrosis in the liver parenchyma respectively.

Biodistribution studies

Biodistribution study was carried out to see the distribution pattern of andrographolide in various organs of the body. At different time interval animal were sacrificed and different organs were isolated to determine the amount of drug reached to organ **(Mishra P. et al. 1991)**. It was found that maximum concentration in liver was reached in 15 mins through oral route. Through administration by oral route the drug reaching stomach was maximum throughout the study which showed a positive result. It is clearly shown in the **(Figure 6)** that

the maximum uptake of the formulation was seen in the liver followed by lungs, kidney and spleen etc. these value shows that the formulation was reaching the target site i.e. liver.

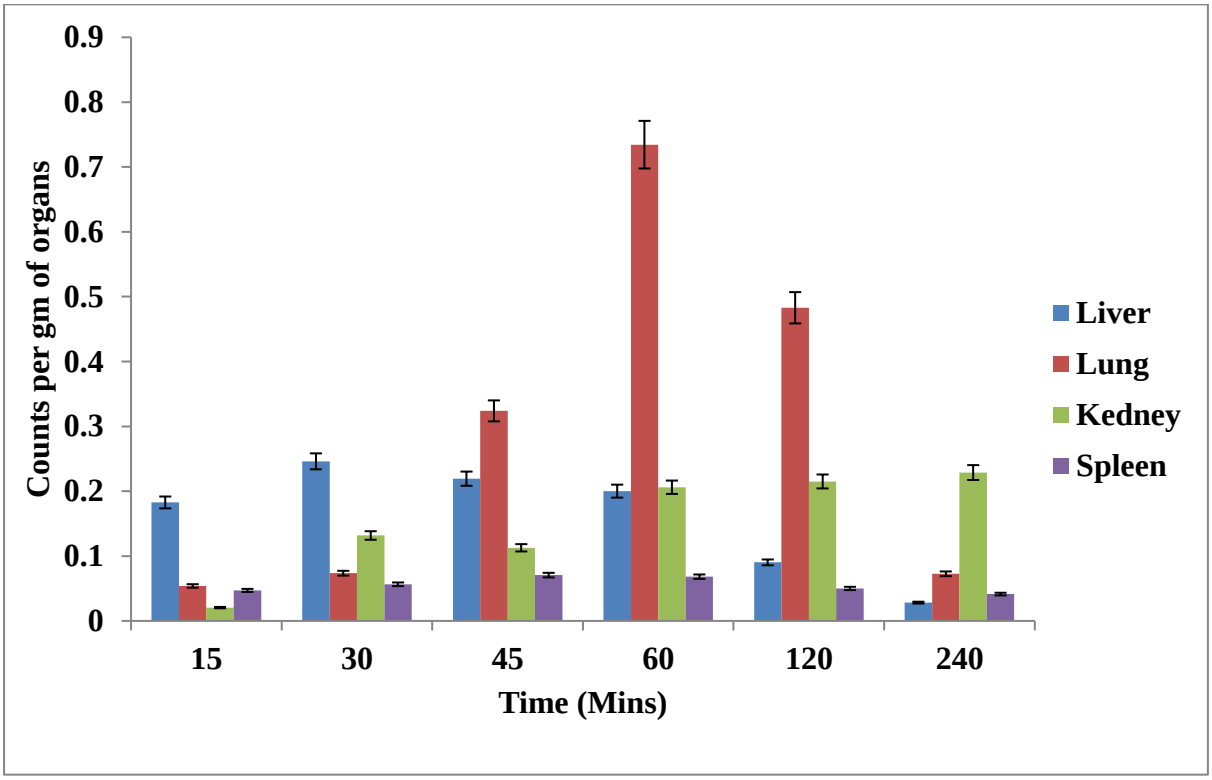

Figure 6: Biodistribution of radiolabelled G-AND-SLNs in different animal organs

Stability studies

Finally stability of the glycyrrhetinic acid mediated andrographolide loaded solid lipid nanoparticles formulations was studied at 40 ± 1 °C and 70 ± 1 % RH for duration of three months as per the ICH guidelines. 3 Formulations were slightly turned to yellowish and light yellow colorations upon standing when compared to samples kept initially **(Kim K.S. et al. 2017)**. All formulations were viscous oily odorless, transparent and of thick liquid consistency. However assay of andrographolide in developed formulations are needed to be determined along with its droplet size and drug permeation characteristics **(Lei Y. et al. 2012)**.

Conclusion

The results indicated that encapsulation of andrographolide in solid lipid nanoparticles improvement in the residence time as well as drug concentration in the 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; MDA: Malondialdehyde; GSH: Reduced Glutathione; i.p: intraperitoneal; i.v: intravenous; 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 liver disease and liver treatments approaches and were the most important contribution in making the manuscript. SK contributed in the animal experimental work. NS elaborated the formulation part in the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS- We are thankful to the management of Faculty of Pharmacy, Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India, for providing the necessary laboratory, animal house, library and internet facilities for the completion of this Research manuscript.

REFERENCES

1. 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.
2. 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
3. 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

4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. Ellman G.L. 1959. Tissue sulfhydryl groups. Arch Biochem Biophys. 82 (1), 70-77.
10. Gupta, S., Sharma, U., Jagannathan, N. R., & Gupta, Y. K. (2017). Neuroprotective effect of lercanidipine in middle cerebral artery occlusion model of stroke in rats. Experimental Neurology, 288, 25–37.
11. Hung MY, Fu TY, Shih PH, Lee CP, Yen GC. Du-Zhong (Eucommia ulmoides Oliv) leaves inhibit CCl4-induced hepatic damage in rats. Food Chem Toxicol 2006;44: 1424–31.
12. 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.
13. 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.

14. 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.
15. 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.
16. 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.
17. 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.
18. Kawakami S et al. Asialoglycoprotein receptor-mediated gene transfer using novel galactosylated cationic liposomes. Biochem Biophys Res Commun 1998; 252: 78–83.
19. Kim, K. S., Yang, E. S., Kim, D. S., Kim, D. W., Yoo, H. H., Yong, C. S., … Choi, H.G. (2017). A novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for improved stability and oral bioavailability of an oily drug, 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol. Drug Delivery, 24(1), 1018–1025.
20. 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
21. 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.
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. L. Lomlim, N. Jirayupong, and A. Plubrukarn, "Heataccelerated degradation of solid-state andrographolide," Chemical & Pharmaceutical Bulletin, vol. 51, no. 1, pp. 24–26, 2003.

24. 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.
25. 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.
26. Lei, Y., Qi, J., Nie, S., Hu, F., Pan, W., Lu, Y., & Wu, W. (2012). Solid Self-Nanoemulsifying Cyclosporine A Pellet Prepared by Fluid-Bed Coating: Stability and Bioavailability Study. Journal of Biomedical Nanotechnology, 8(3), 515–521.
27. 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.
28. 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
29. Mahajan H, Shamshadbeldar. Rutin loaded nanoemulsion formulation for brain tumor targeting: In vitro, ex vivo permeation and in vitro cytotoxicity assay. Indian Journal of Novel Drug Delivery 9(2), 2017, 96-106.
30. 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.
31. Mishra D.,et al. Glycyrrhizin conjugated chitosan nanoparticles for hepatocyte-targeted delivery of lamivudine. Journal of Pharmacy and Pharmacology. 2014; 1-12.
32. Mishra P., Babbar A., Chauhan U.P. (1991). A rapid instant thin layer chromatographic procedure for determining radiochemical purity of 99Tcm-IDA agents. Nuclear Medicine Communications, 12(5), 467–468.
33. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351–358.

34. 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.
35. P. K. Singha, S. Roy, and S. Dey, "Antimicrobial activity of Andrographis paniculata," Fitoterapia, vol. 74, no. 7-8, pp. 692 – 694, 2003.
36. Pangeni, R., Sharma, S., Mustafa, G., Ali, J., & Baboota, S. (2014).Vitamin E loaded resveratrol nanoemulsion for brain targeting for the treatment of Parkinson's disease by reducing oxidative stress. Nanotechnology, 25(48), 485102.
37. Placer, Z. A., Cushman, L. L., & Johnson, B. C. (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical Biochemistry, 16(2), 359–364.
38. 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.
39. 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.
40. 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.
41. Rechnagel RO, Glende Jr EA. Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. CRC Crit Rev Toxicol 1973; 2:263–97.
42. 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.
43. 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

44. 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.
45. 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.
46. V. J. Ram, "Herbal preparations as a source of hepatoprotective agents," Drug News and Perspectives, vol. 14, no. 6, pp. 353–363, 2001.
47. 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
48. 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
49. 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.
50. 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