Arabian Journal of Chemistry (2020) 13, 3682–3689

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Enhancement of bioavailability and hepatoprotection by silibinin through conversion to nanoparticles prepared by liquid antisolvent method

Muhammad Umar Khayam Sahibzada [a], Abdul Sadiq [b], Muhammad Zahoor [c,*], Sumera Naz [c], Muhammad Shahid [a], Najam Afaq Qureshi [a]

[a] *Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar 26000, KPK, Pakistan*
[b] *Department of Pharmacy, University of Malakand, Chakdara, Lower Dir 18800, KPK, Pakistan*
[c] *Department of Biochemistry, University of Malakand, Chakdara, Lower Dir, KPK 18800, Pakistan*

Received 2 November 2019; accepted 5 January 2020
Available online 13 January 2020

KEYWORDS

Silibinin;
Pharmacokinetic parameters;
Carbon tetrachloride;
Hepatoprotective activity

Abstract The current research was intended to establish the impact of Silibinin nanoparticles (SB-APSP) produced by the antisolvent precipitation with a syringe pump (APSP). The *in-vivo* bioavailability and hepatoprotective activity of SB-APSP were evaluated in experimental animals. To determine the pharmacokinetic parameters, silibinin and its nanoparticles were given orally to rabbits at a dose of 50 mg/Kg body weight. Blood samples were drawn at different time intervals and were analyzed using HPLC. The bioavailability of un processed silibinin was lower as compared to silibinin nanoparticles (3.45 ± 0.07 and 23.76 ± 0.07 µg/mL respectively). The AUC and C_{max} of SB-APSP were found to be 15.56 and 6.88 folds greater for nanoparticles when compared to silibinin. Hepatoprotective study in Male Sprague Dawley rats revealed that SB-APSP provide better recovery of the damaged liver cell induced by CCl_4. Histopathology of the liver revealed that SB-APSP provide better protection to the liver cells from the damage induced by CCl_4 and maintained the hepatic lobule histopathology more efficiently. It was concluded that the SB-APSP can more effectively protect the liver in experimental animals in a far better way compared to the un-processed Silibinin and could be used as an efficient hepatoprotective agent.

1. Introduction

Liver is the key site where different kind of xenobiotics, environmental pollutants and most importantly the chemotherapeutic agents gets metabolized and is thus exposed to infections by a number of agents (Amirsaadat et al., 2017; Dixit et al., 2007; Hussain et al., 2011; Loguercio and Festi, 2011). Liver has its own defense mechanism which protects it

* Corresponding author.
E-mail address: mohammadzahoorus@yahoo.com (M. Zahoor).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2020.01.002
1878-5352

from the harmful effects of different compounds that get metabolized there. The Failure of this defense system will make liver susceptible to the attack of microbes and toxic chemical which ultimately would result into liver atrophy. Liver diseases are prevalent worldwide and many scientists are working to develop new compounds having the ability to cure the damaged liver. A number of starategies have been developed to treat liver diseases. Although synthetic drugs are quite effective but associated with many undesirable side effects. The use of natural antioxidants and hepatoprotective plant products are considered to be the best alternative of synthetic hepatoprotective drugs as they have a natural origin and exhibits very few side effects (Dixit et al., 2007). Silymarin, a herbal formulation has shown hepatoprotective potentials in liver damage. Chemically, it is a flavonolignane complex containing silibinin and its isomers; silicristin and silidianin. About 60% of silymarin is silibinin while 40% are the other two isomers. As silymarin main component is silibinin which is sparingly soluble in water which thus reducing the efficacy of the formulation in liver damage. A number of attempts have been made to increase the solubility of silibinin in aqueous media (Woo et al., 2007; Wu et al., 2007; Wu et al., 2009).

Amongst the different approaches used to enhance the solubility of silibinin and thus bioavailability; complexation and solid dispersions are considered to be the most effective techniques that considerably increase the bioavailability (Arcari et al., 1992; Barzaghi et al., 1990; Chen et al., 2005; Ellahi et al., 2019; Koo et al., 2002; Morazzoni et al., 1992; Prakash et al., 2019). Although enhancing bioavailability through dispersion is effective techniques but if a drug component to be dispersed have particle sizes in micro range would not be much effective in comparison to nano size particles. Smaller size particles have larger surface area and high proportion of surface atoms. Nanotechnology is an emerging field of science that deals with conversion of larger particles into smaller one having diameter in nanometers range. Sparingly soluble substances having particles in nanorange can more effectively be dispersed in the aqueous medium in comparison to particles having size microrange (Javed et al., 2011; Jogaiah et al., 2019; Palencia et al., 2019; Zeeshan et al., 2018).

A number of approaches have been used to convert sparingly soluble substances into nano sized particles. Amongst them solvent/antisolvent approach have produced far reaching results whereby an antisolvent is added to the saturated solution of the sparingly soluble substance to produce nanoparticles in the form of precipitates. The precipitation method has advantage over other approaches as they requires low energy and are simple, and robust. Also the nanoparticles fabricated by the precipitation methods have more surface energy and have fine and uniform particle size (Sahibzada et al., 2017).

Although silibinin nanoparticles have been synthesized by a number of methods, however, the solvent/antisolvent method have not been used to prepare its nanoparticles. Therefore, in the current study the approach of antisolvent precipitation with a syringe pump (APSP) was used to prepare silibinin nanoparticles to enhance its bioavailability. The evaluation of pharmacokinetic parameters and consequently its hepatoprotective ability was checked in experimental animals (Rabbits and Male Sprague Dawley rats).

2. Experimental

2.1. Materials

All the chemicals utilized in the current study were of analytical grade with highest purity. Silibinin (98% pure) and Silymarin were acquired from the Sigma-Aldrich (St Louis, MO, USA). Standard histotechnology procedures were adopted for the preparation of laboratory reagents like Harris hematoxylin, eosin stain and neutrally buffered formalin solution (10%). (Prophet et al., 1992) HPLC system used during the bioavailability studies was Perkin Elmer Series 200 HPLC system. Healthy rabbits each weighing 2–3 kg were selected for bioavailability studies. For hepatoprotective activity, Male Sprague Dawley rats, each weighing 150–200 gm, were acquired from the National Institute of Health (NIH), Islamabad. The experimental animals were acclimatized at 22 ± 2 °C for seven days prior to use. Animals were given food and water *ad libitum*. All the procedures on experimental animals were carried out according to the ARRIVE and NIH Animal Research guidelines. The current study was approved by the Ethical Committee of the University of Malakand.

2.2. Preparation of nanoparticles

Nanoparticles from the unprocessed silibinin (SB) were fabricated according to the method reported by Sahibzada *et al.* (2017). The Antisolvent precipitation with a syringe pump technique was utilized for the nanoparticle fabrication as shown in Fig. 1. SB solution was injected at constant rate and stirring to the stabilizer solution which was composed of propylene glycol and water, acted as antisolvent. The prepared nanosuspension was subjected to the rotary evaporator under vacuum to yield solid nanoparticles (SB-APSP). Different analytical techniques like particle size analyzer, SEM, XRD and

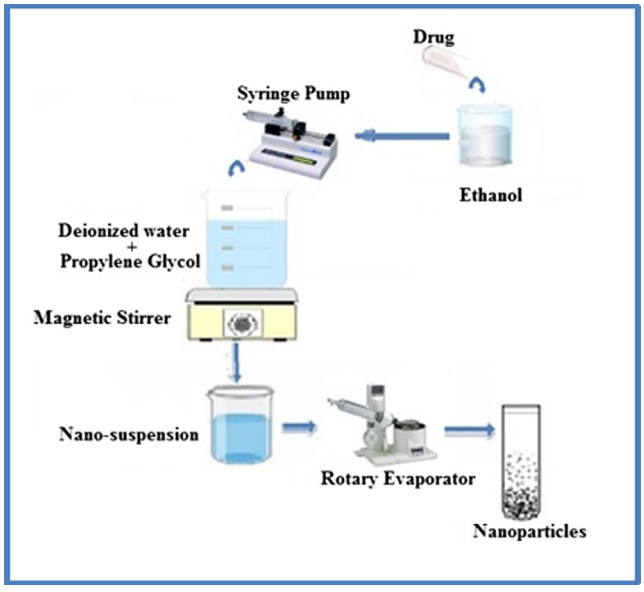

Fig. 1 Preparation of naoparticles.

DSC were used to characterize the nanoparticles (Bhatti et al., 2018; Jogaiah et al., 2019; Palencia et al., 2019; Sarafraz et al., 2020).

2.3. Bioavailability studies

To determine the bioavailability of prepared nanoparticles and Silibinin, two groups of healthy rabbits (7 rabbits each) were made. One was given unprocessed drug while to the other group, the nanoparticles. Experimental animals were fasted for 12 h prior to the experiment. Nano drug was administered orally at a dose of 50 and 100 mg/kg of body weight to the animals. About 2 mL of blood was drawn from the marginal ear vein of each animal at predetermined time intervals (0, 30, 60, 90, 120, 240, 360, 720 and 1440 min). The blood samples were instantly centrifuged for 20 min at 3,000 RPM. The supernatant was separated and stored in frozen state until further use. The samples were analyzed through HPLC following the procedure described Wu *et al* (2007).

2.4. Hepatoprotective study

To determine the bioavailability of prepared nanoparticles and Silibinin, Six groups of healthy Male Sprague Dawley rats (n = 7) were made. Each animal was administered both SB 200 mg/kg and SB-APSP (used at a dose of 50 mg/kg and 100 mg/Kg) both suspended in CMC solution (0.5% w/v). The medications were administered through an oral gavage tube for three weeks as shown in Fig. 2. As positive control, Silymarin at a dose of 200 mg/kg, suspended in 1% CMC was utilized. A saline solution containing 0.5% w/v carboxy methyl cellulose was used as negative control. At the end of dose regimen, a single 2 mL/kg dose of carbon tetrachloride (CCl_4 in olive oil) was administered intraperitoneally, two hours after the administration of drugs. The experimental animals were distributed into groups as shown according to the plan shown in Table 1.

2.4.1. Blood biochemical investigations

From the experimental animals, blood samples were collected 24 h after the CCl_4 treatment and were allowed to clot following by centrifugation (K 240 R, Centurion scientific, UK) for 15 min at 3000 rpm, to separate serum, which was kept in storage at 4 °C till further use. The biochemical parameters determined were liver function test (LFT) enzymes levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined (GO F400 CH, Chema Diagnostica, Italy).

2.4.2. Histological evaluation

At the end of dose regimen, liver samples were taken from each rabbit and were put in neutrally buffered formalin solution (10%) for 48 h followed by dehydration of the liver tissues in ethanol solution. Finally, the liver tissues were cleared and permeated in two changes, each of 100% xylene. Liver tissues were then placed in paraffin wax and were sectioned into tissue blocks of 4 μm size using a rotary microtome. During microscopic observation, the tissue blocks were stained with Harris hematoxylin and eosin (H&E) dye solution.

2.5. Statistical data analysis

The experimental data was analyzed by one way ANOVA, which was followed by Tukey's multiple comparison *post hoc* test to determine the data statistical significance of the differences between groups.

3. Results and discussion

Nanoparticles of the Silibinin were obtained using antisolvent precipitation method with a syringe pump. To get nanoparticles of optimum particle size and PDI a number of experiments were carried out. Optimally sized SB-APSP were obtained at stirring speed 3000 rpm, Solvent-antisolvent ratio (1:10) and

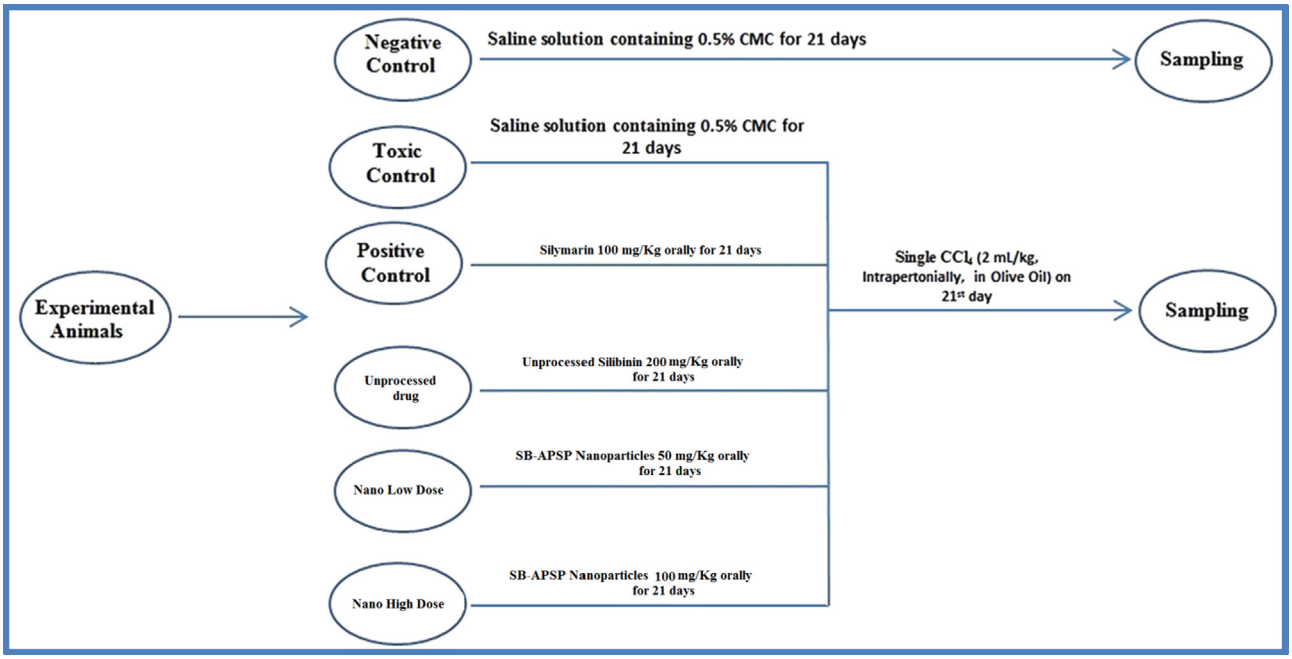

Fig. 2 Experimental animals dosing scheme.

Table 1 Treatment groups for the hepatoprotective study.

Groups	Drug administered
1st Group	Negative control
2nd Group	Carbon tetrachloride
3rd Group	Silymarin
4th Group	Unprocessed silibinin-200
5th Group	SB-APSP-50
6th Group	SB-APSP-100

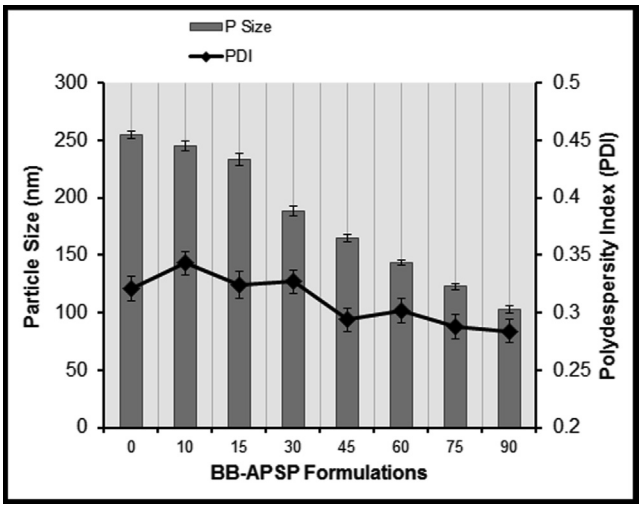

Fig. 3 Optimization of Particle size and PDI for APSP method; BB-APSP.

1% w/v concentration of the propylene glycol. The size of SB-APSP at optimized condition obtained were: 104.5 ± 3.2 nm and the PDI: 0.301 ± 0.02 (Fig. 3). Particle size reduction results in greater surface area and have diffusion layer with less thickness. Both of them improve the contact of solvent with the material that help in improving the solubility, dissolution and bioavailability (Sahibzada et al., 2018). SEM data confirmed the nano size of the prepared nanoparticles (Fig. 4). XRD and DSC analysis also confirmed less crystalline structure of the nanoparticles when compared to the unprocessed drug (Figs. 5 and 6).

3.1. Bioavailability studies

Bioavailability studies revealed that the prepared nanoparticles have a better pharmacokinetic profile in comparison to the unprocessed drug. Following administration orally, the AUC and C_{max} for SB-APSP were found to be 15.6 and 6.9 times greater than the unprocessed Silibinin (Fig. 7 and Table 2).

Previous studies revealed that Silibinin have limited bioavailability, which is linked to the poor absorption rate and high reactivity with phase II conjugation (Lorenz et al., 1984; Wu et al., 2007). On the other hand, when the Silibinin was converted to nano form by APSP method resulted in fortified bioavailability and improved pharmacokinetic profile. The pharmacokinetic evaluation data in Table 2 revealed that the plasma level of un-processed silibinin was very low compared to the nanoparticles. The plasma concentration of SB and SB-APSP recorded during the study were 3.45 ± 0.07 and 23.76 ± 0.07 μg/mL, respectively. Particle size reduction enhanced the surface area, reduced thickness of the diffusion layer, improved adhesion of the material to the cell membrane and also caused an increase in the surface free energy which improved its solubility and bioavailability.

3.2. Hepatoprotective studies

Silibinin constitute about 60% of the silymarin, so silibinin has a major role in the silymarin hepatoprotective activity and all other benficial effects. Hepatoprotective activity of silymarin has been demonstrated by various researchers from all over the world in experimental animals by using carbon tetrachloride as toxicant (Mourelle et al., 1989; Muriel and Mourelle, 1990). Among other effects, it prevents the increase in liver lipoperoxidation caused by CCl_4. The silibinin beneficial effects are due to its capability to inhibit lipoperoxidative processes, since lipid peroxidation is one of the main factor of $CC1_4$ induced liver damage. $CC1_4$ metabolism results in the Free radicals which attack and modify the membrane composition and thus alters the activities of certain ATPases (plasma membrane-embedded enzymes). Steatosis could result from a combination of reduced apolipoprotein production, enhanced free fatty acid influx, and maybe decreased fatty acid beta-oxidation in the hepatocytes. In addition, the ability of silymarin to capture free radicals arising from CCl_4 metabolism could prevent the inactivation of those enzymes involved in

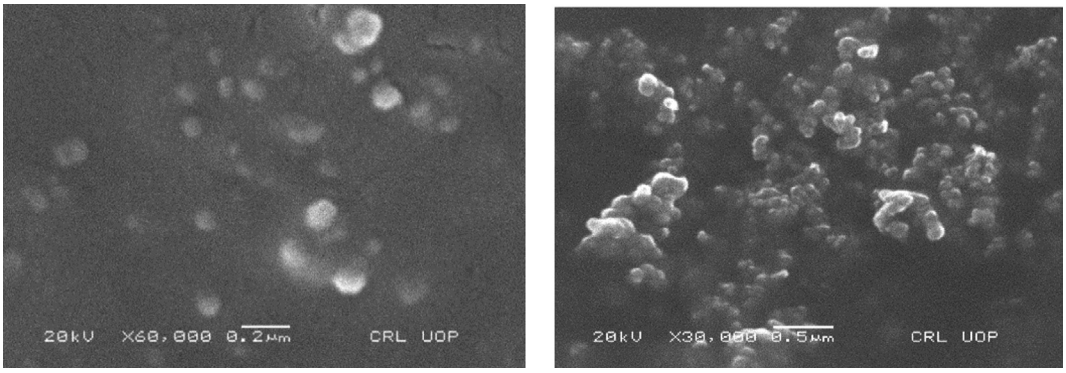

Fig. 4 SEM pictures of SB-APSP with different magnifications.

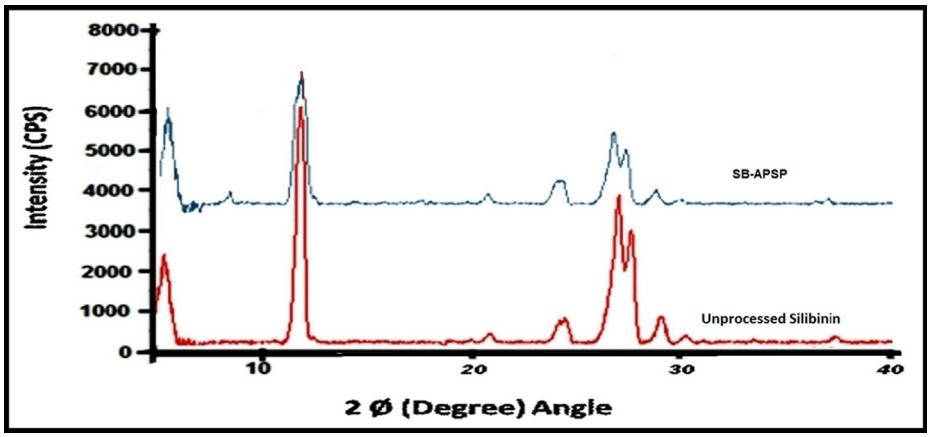

Fig. 5 XRD diffractogram of SB and SB-APSP.

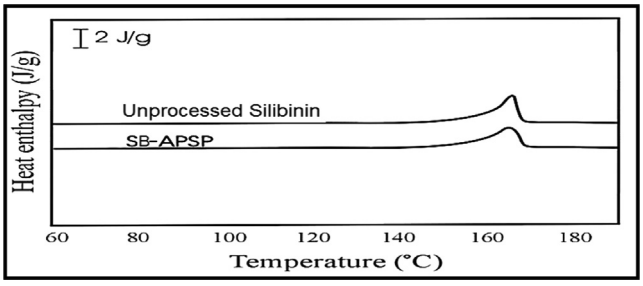

Fig. 6 DSC results of APSP method.

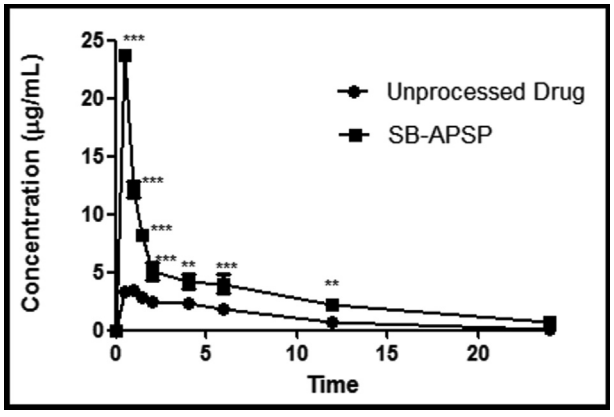

Fig. 7 Graphical representation of the In-vivo studies for bioavailability assessment.

beta-oxidation of fatty acids (Mourelle et al., 1989). The observations recorded in this study are described in following lines:

3.2.1. Biochemical evaluation of the silibinin and its nanoparticles

To evaluate the hepatoprotective effect of unprocessed Silibinin and their nanoparticles (SB-APSP), the experimental animals were intoxicated with carbon tetrachloride (CCl_4) to produce hepatotoxicity (Bhathal et al., 1983). Certain biochemical parameters like ALT, AST, ALP, and histopathological evaluation were carried out to assess the hepatotoxicity caused by the CCL_4 which resemble histologically to the viral hepatitis (James and Pickering, 1976). As shown in Fig. 8, substantial alterations in serum LFT enzymes levels of the groups

Table 2 In-vivo bioavailability studies of silibinin and its nanoparticles.

Samples	Pharmacokinetic parameter		
	T_{max} (Hours)	C_{max} (µg/mL)	$AUC_{0\text{-}t}$ (µg-h/mL)
Unprocessed silibinin	1.0 ± 0.15	3.45 ± 0.07	20.48 ± 3.16
Nanoparticles	0.5 ± 0. 11	23.76 ± 0.07	318.63 ± 12.64

Values are expressed as mean ± SD. $n = 6$ Rabbits per sample.

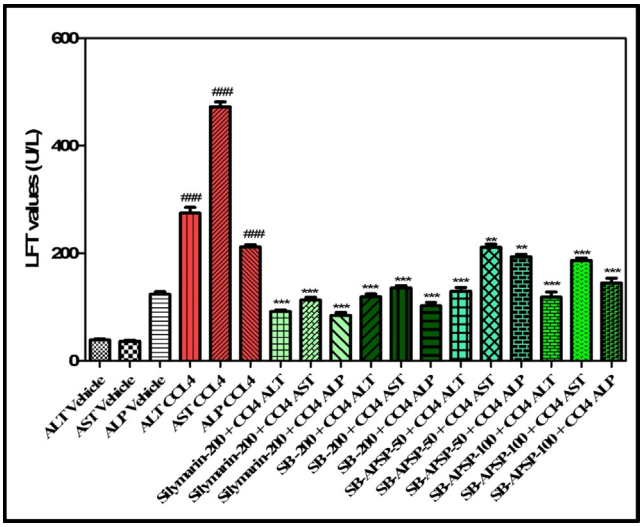

Fig. 8 Serum biochemical evaluation of the LFT enzymes in different treatment groups. Values presented as mean ± SEM. $^{###}P < 0.001$ compared to control vehicle alone treated group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to CCL_4 alone treated group. One way ANOVA followed by *post hoc* Tukey's multiple comparison test ($n = 7$ rats per group).

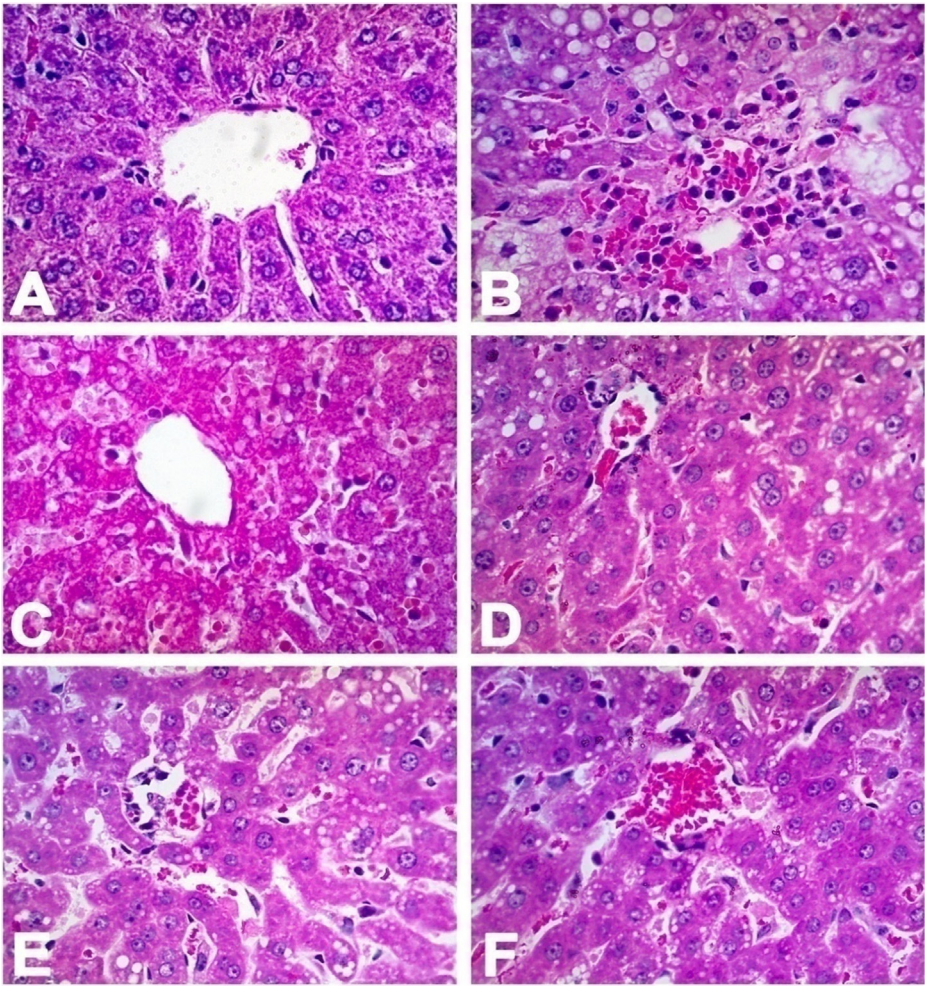

Fig. 9 Histopathological assessment of hepatotoxicity studies (H & E; x400 original magnification) (n = 7 rats per group).

treated with silymarin, unprocessed Silibinin and SB-APSP were observed; AST [$F(7,40) = 21.01$, $P < 0.0001$], ALP [$F(7,40) = 7.158$, $P < 0.0001$] and ALT [$F(7,40) = 25.18$, $P < 0.0001$] following the CCL_4 dose. A comparison with the control showed that CCL_4 caused an enormous and significant elevation ($P < 0.001$) of LFT enzymes levels. On the other hand, the groups treated with the Silibinin nanoparticles at doses of 50 mg/kg ($P < 0.01$) and 100 mg/kg ($P < 0.001$) compensated the resultant increase in the LFT enzymes levels induced by the CCL_4 to a greater extent. The positive control, silymarin which was administered at a dose of 200 mg/kg, have remarkably counteracted ($P < 0.001$) the serum LFT enzymes levels of the treatment group that received unprocessed Silibinin at a dose of 200 mg/Kg.

3.2.2. Histopathological evaluation

The hepatic tissues isolated from different treatment groups were evaluated histopathologicaly to evaluate the impact of different medication on the CCL_4 induced hepatotoxicity. In a study conducted by Akbari-Kordkheyli et al. induced liver ischemia-reperfusion injuries (I/RI) showed that Silibinin inhibited hepatocyte vacuolization and degeneration, endothelium damages, sinusoidal congestion and inflammation during I/RI (Akbari-Kordkheyli et al., 2019). In the current study, the treated groups received CCl_4 showed the extensive necrosis that can be seen throughout the hepatic lobule. In the Fig. 9, we can see that the central vein is damaged badly and is collapsing due to the necrosis caused by the CCl_4. The central vein and dilated sinusoidal space are covered with the debris of red blood cells, lymphocytes shown as blue dots and many necrotic fragments that are spread all over. Also the lipid peroxidation induced by the CCl4 can be seen in the form of microvesicular and macrovesicular steatosis. Intense coagulative necrosis along with the hyaline inclusions in the Centrilobular hepatocytes can be seen in Fig. 9B.

Fig. 9 shows hepatic tissues photomicrograph of different groups. The first photomicrograph (9A) represents the group treated with saline solution only; negative control, in which the central vein appears to have normal physiology with proper boundaries. The hepatocytes plates appear to normal with clear sinusoidal space visible. Fig. 9B represent the animal group treated with toxic control; CCl_4. The central vein appears to be collapsed/congested and covered with the debris of red blood cells, lymphocytes (blue dots) and necrotic fragments, spread throughout the photomicrograph. Hepatocytes have severe necrosis along with the macrovesicular and microvesicular steatosis because of lipid peroxidation induced by the CCl_4. Liver cross section shown in Fig. 9C belongs to

the group treated with silymarin, 200 mg. One can clearly see that the liver has maintained its normal physiology and not much damage has occurred due to CCl_4. Fig. 9D belongs to the group treated with unprocessed drug administered at a dose of 200 mg with mild microvesicular steatosis. Fig. 7E and F represent the liver cross section obtained from the animal treated with silibinin nanoparticles given at a dose of 50 and 100 mg respectively. One can see that sinusoidal dilatation and some mild microvesicular steatosis yet the extent of damage from the CCL_4 has been prevented to a greater extent by the nanoparticles and have preserved the hepatic lobule basic histoarchitecture yet there was central veins congestion but within a reasonable limit, permeation of red blood cells and lymphocytes into sinusoidal spaces which were dilated, also mild microvesicular steatosis and infrequent macrovesicular steatosis. Pretreatment with Silibinin that have anti inflammatory and anti-free radicals properties explain the protection caused by the drug (Akbari-Kordkheyli et al., 2019; Tsai et al., 2008). Silibinin nanoparticles treatment reduced the severity of hepatic damage, when compared to that observed results for the unprocessed drug after CCl_4 treatment. The decreased neutrophil infiltration in the hepatic tissue further indicated its significant hepatoprotective effect when compared to the similar studies conducted by other researchers (Akbari-Kordkheyli et al., 2019; Freitag et al., 2015).

Alteration in the endoplasmic reticulum were the beginning of liver toxicity which resulted in the loss of metabolic enzymes located in the intracellular structures (Jain et al., 2008; Recknagel, 1983). Also a toxic metabolite carbon trichloride (CCl_3) radical is produced after the ingestion of CCL_4 which further convert to trichloromethyl per-Oxy radical after reaction with the oxygen by the cytochrome P450 2E1 enzyme. Trichloromethyl per-oxy radical then bind covalently to the macromolecules and causes the per-oxidative degradation of adipose tissue lipid membrane. Silibinin causes the reduction of these effects by reducing the levels of hepatic enzymes like ALT and AST, which were enhanced upon conversion to the nano form. Reduction in the levels of hepatic enzymes is an indication of the stabilization of plasma membrane and also an indication of the recovery of liver tissues from the damage caused by CCL_4. The recovery of hepatic parenchyma and rejuvenation of hepatocytes start as the serum levels of transaminases return back to normal (Thabrew et al., 1987). A little microvesicular steatosis was the only histopathological alteration that was observed in the animal groups, treated with silibinin or silymarin (200 mg/kg) plus carbon tetrachloride (Fig. 9C and 9D). Silibinin nanoparticles performed effectively in treating the CCL_4 induced toxicity comparative to the unprocessed silibinin and silymarin which has established hepatoprotective effects (Vargas-Mendoza et al., 2014).

4. Conclusion

The hepatoprotective effects and pharmacokinetic profiles produced by the silibinin nanoparticles were found better when compared to that of un-processed silibinin. Maximum plasma concentration values and AUC resulted from the nanoparticles were better than that of Un-processed drug. The fabricated nanoparticles could regularize the hepatic cytochrome P450 enzyme system and stimulate the liver regenerative activity as they restored all the liver function markers. Histological micrographs of liver sections indicated the diminution of liver impairment induced by CCL_4. The data obtained in this study clearly revealed that nanoparticles of silibinin effectively enhance the therapeutic effects.

5. Authorship declaration

All the contributing authors were equally involved in this piece of research. The article was finalized with the approval of all authors.

6. Disclosure statement

The authors declare no conflict of interests.

Author contributions

"S.M.U.K and A.S. conceived and designed the experiments; S.M.U.K., M.Z, M.S. and S.A. performed the experiments; S.M.U.K, M.Z., S.A., M.S. and N.A.Q. analyzed the data; A.S. and M..Z. contributed reagents and materials; All the authors contributed equally in the paper write up.

References

Akbari-Kordkheyli, V., Azizi, S., Khonakdar-Tarsi, A., 2019. Effects of silibinin on hepatic warm ischemia-reperfusion injury in the rat model. Iran. J. Basic Med. Sci. 22, 789–796.

Amirsaadat, S. et al, 2017. Silibinin-loaded magnetic nanoparticles inhibit hTERT gene expression and proliferation of lung cancer cells. Artificial cells. Nanomed. Biotechnol. 45, 1649–1656.

Arcari, M. et al, 1992. A new inclusion complex of silibinin and beta-cyclodextrins: in vitro dissolution kinetics and in vivo absorption in comparison with traditional formulations. Boll. Chim. Farm. 131, 205–209.

Barzaghi, N., Crema, F., Gatti, G., Pifferi, G., Perucca, E., 1990. Pharmacokinetic studies on IdB 1016, a silybin-phosphatidylcholine complex, in healthy human subjects. Eur. J. Drug Metab. Pharmacokinet. 15, 333–338.

Bhathal, P., Rose, N., Mackay, I., Whittingham, S., 1983. Strain differences in mice in carbon tetrachloride-induced liver injury. Br. J. Exp. Pathol. 64, 524.

Bhatti, M., Zeeshan, A., Tripathi, D., Ellahi, R., 2018. Thermally developed peristaltic propulsion of magnetic solid particles in biorheological fluids. Ind. J. Phys. 92, 423–430.

Chen, W., Xia, H., Wu, W., 2005. Optimized preparation of silymarin dripping pills by a central composite design response surface method. Chin Trad Herb Drug 36, 679–683.

Dixit, N., Baboota, S., Kohli, K., Ahmad, S., Ali, J., 2007. Silymarin: a review of pharmacological aspects and bioavailability enhancement approaches. Ind. J. Pharmacol. 39, 172.

Ellahi, R., Zeeshan, A., Hussain, F., Asadollahi, A., 2019. Peristaltic blood flow of couple stress fluid suspended with nanoparticles under the influence of chemical reaction and activation energy. Symmetry 11, 276.

Freitag, A.F. et al., 2015. Hepatoprotective effect of silymarin (Silybum marianum) on hepatotoxicity induced by acetaminophen in spontaneously hypertensive rats. Evid.-Based Complementary Altern. Med. 2015.

Hussain, I., Khan, F.A., Ullah, R., Khan, N., Muhammad, Z., 2011. Analysis of silymarin in the seeds of white and blue capitulum of Silybum marianum plants. Afric. J. Pharm. Pharmacol. 5, 742–745.

Jain, A. et al, 2008. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioica Roxb. leaves. J. Ethnopharmacol. 115, 61–66.

James, G., Pickering, R., 1976. The protective effect of a novel compound RU 18492, on galactosamine-induced hepatotoxicity in the rat. Arzneimittelforschung 26, 2197–2199.

Javed, S., Kohli, K., Ali, M., 2011. Reassessing bioavailability of silymarin. Alt. Med. Rev. 16, 239.

Jogaiah, S., Kurjogi, M., Abdelrahman, M., Hanumanthappa, N., Tran, L.-S.P., 2019. Ganoderma applanatum-mediated green synthesis of silver nanoparticles: Structural characterization, and in vitro and in vivo biomedical and agrochemical properties. Arab. J. Chem. 12, 1108–1120.

Koo, C., Choi, D., Song, I.-Y., 2002. Compositions and preparations of silymarin complex with improved bioavailability. WO 2, 069962.

Loguercio, C., Festi, D., 2011. Silybin and the liver: from basic research to clinical practice. W. J. Gastroenterol. 17, 2288.

Lorenz, D., Lücker, P., Mennicke, W., Wetzelsberger, N., 1984. Pharmacokinetic studies with silymarin in human serum and bile. Methods Find. Exp. Clin. Pharmacol. 6, 655–661.

Morazzoni, P., Magistretti, M., Giachetti, C., Zanolo, G., 1992. Comparative bioavailability of silipide, a new flavanolignan complex, in rats. Eur. J. Drug Metab. Pharmacokinet. 17, 39–44.

Mourelle, M., Muriel, P., Favari, L., Franco, T., 1989. Prevention of CCl4-induced liver cirrhosis by silymarin. Fundam. Clin. Pharmacol. 3, 183–191.

Muriel, P., Mourelle, M., 1990. Prevention by silymarin of membrane alterations in acute CCI4 liver damage. J. Appl. Toxicol. 10, 275–279.

Palencia, M., Córdoba, A., Melendrez, M.F., 2019. Nanocomposites based on cationic polyelectrolytes and silver nanoparticles: Synthesis, characterization, molybdate retention and antimicrobial activity. Arab. J. Chem. 12, 825–834.

Prakash, J., Tripathi, D., Tiwari, A.K., Sait, S.M., Ellahi, R., 2019. Peristaltic pumping of nanofluids through a tapered channel in a porous environment: applications in blood flow. Symmetry 11, 868.

Prophet, E.B., Mills, B., Arrington, J.B., Sobin, L.H., 1992. Laboratory methods in histotechnology. American Registry of Pathology Washington, DC.

Recknagel, R.O., 1983. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci. 33, 401–408.

Sahibzada, M.U.K. et al, 2018. Berberine nanoparticles with enhanced in vitro bioavailability: characterization and antimicrobial activity. Drug Des. Devel. Ther. 12, 303.

Sahibzada, M.U.K., Sadiq, A., Khan, S., Faidah, H.S., 2017. Fabrication, characterization and in vitro evaluation of silibinin nanoparticles: an attempt to enhance its oral bioavailability. Drug Des. Devel. Ther. 11, 1453.

Sarafraz, M., Pourmehran, O., Yang, B., Arjomandi, M., Ellahi, R., 2020.. Pool boiling heat transfer characteristics of iron oxide nanosuspension under constant magnetic field. Int. J. Therm. Sci. 147, 106131.

Thabrew, M.I., Joice, P., Rajatissa, W., 1987. A comparative study of the efficacy of Pavetta indica and Osbeckia octandra in the treatment of liver dysfunction. Planta Med. 53, 239–241.

Tsai, J. et al, 2008. Effects of silymarin on the resolution of liver fibrosis induced by carbon tetrachloride in rats. J. Viral Hepat. 15, 508–514.

Vargas-Mendoza, N. et al, 2014. Hepatoprotective effect of silymarin. W. J. Hepatol. 6, 144.

Woo, J.S., Kim, T.-S., Park, J.-H., Chi, S.-C., 2007. Formulation and biopharmaceutical evaluation of silymarin using SMEDDS. Arch. Pharmacal Res. 30, 82–89.

Wu, J.-W., Lin, L.-C., Hung, S.-C., Chi, C.-W., Tsai, T.-H., 2007. Analysis of silibinin in rat plasma and bile for hepatobiliary excretion and oral bioavailability application. J. Pharm. Biomed. Anal. 45, 635–641.

Wu, J.-W., Lin, L.-C., Tsai, T.-H., 2009. Drug–drug interactions of silymarin on the perspective of pharmacokinetics. J. Ethnopharmacol. 121, 185–193.

Zeeshan, A., Ijaz, N., Abbas, T., Ellahi, R., 2018. The sustainable characteristic of bio-bi-phase flow of peristaltic transport of MHD Jeffrey fluid in the human body. Sustainability 10, 2671.