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Potential therapeutic effects of silymarin and silymarin-loaded solid lipid nanoparticles on experimental kidney damage in BALB/c mice: biochemical and histopathological evaluation

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Abstract: Silymarin (Sm) is widely used in treating diseases that affect organs such as the liver, kidney, and gallbladder thanks to its antioxidative, renoprotective, antihepatotoxic, and anticarcinogenic properties. However, this substance is poorly solved in water and tends to decompose in the intestine, its bioavailability decreasing before it can show real effect. With these limitations in mind, the present study aims to enhance the poor bioavailability of Sm by forming Sm-loaded solid lipid nanoparticles (Sm-SLNs) using the hot homogenization method. A characterization process was undertaken to determine possible impact of Sm on experimental kidney damage. Our biochemical and light microscopic results suggest that the group that received Sm-SLNs for the treatment of D-GalN/TNF-α–induced experimental kidney damage showed significantly more improvement than the group that received commercially available Sm. In conclusion, Sm-loaded SLN may be a useful system for the delivery of poorly water-soluble Sm and may also provide renoprotection.

Key words: D-GalN/TNF-α, kidney damage, silymarin, solid lipid nanoparticle, renoprotectivity

1. Introduction

Renal dysfunction is a common and serious problem associated with advanced stages of liver disease. Renal failure is widely known to occur spontaneously in most patients suffering from acute liver failure (Ring-Larsen and Palazzo, 1981). Hepatotoxic substances including acetaminophen (Pritchard and Butler, 1989; Bourdi et al., 2002), carbon tetrachloride (CCl_4), lipopolysaccharides (Shi et al., 1998; Morio et al., 2001), D-galactosamine (D-GalN), and tumor necrosis factor alpha (TNF-α) are extensively employed in treating experimentally induced acute liver damage cases (Bradham et al., 1998). Administration of D-GalN/TNF-α in varying doses to induce liver injury is a widely chosen experimental method, and it is used for examining pathogenesis of the liver tissue (Tapalaga et al., 2002). TNF-α is not just a cytokine produced and released in macrophages; it is also released at lower intensity by lymphocytes, mast cells, endothelial cells, fibroblasts, and nerve cells (Bradham et al., 1998). D-GalN is a specific hepatotoxic agent, and it blocks hepatic mRNA transcription by depleting uridine nucleotides metabolically (Leist et al., 1994). Although the effects of sole D-GalN (Sracyn et al., 2004) or TNF-α (Tracey et al., 1986) applications on the kidney can be observed, it remains to be seen what influence a combination of D-GalN and TNF-α exerts upon on this tissue.

Sm has generated promising hepatoprotective effects, not only experimentally but also clinically. Antioxidant and anti-inflammatory properties of Sm may also act as a protective agent against photocarcinogens (Mudit and Santosh, 2010) and nephropathic processes (Kren and Walterová, 2005). Administration of silibin, the major active constituent of silymarin, before and after chemically induced injury has been shown to prevent or reduce nephrotoxic effects. Therefore, it was assumed that Sm may act as a renoprotective agent against nephrotoxic medications (Sonnenbichler et al., 1999). The protective effects of Sm on drug-induced nephrotoxicity have been observed, mainly in experiments done on animals, and renoprotection against cisplatin is most frequently reported. However, due to its poor ability to dissolve in

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water, bioavailability of Sm is rather low (Lorenz et al., 1997; Provinciali et al., 2007). According to pharmacokinetic investigations, only 23%–47% of Sm manages to reach systemic circulation once it has been taken orally (Lorenz et al., 1984; Schulz et al., 1995).

In the present study, Sm-loaded solid lipid nanoparticles (Sm-SLNs) were prepared using the hot homogenization method so that a new kind of Sm-loaded SLN with enhanced bioavailability could be obtained. Herein, renoprotective activities of synthesized Sm-loaded SLN and commercial Sm in D-GalN/TNF-α–induced kidney damage on BALB/c mice were investigated.

2. Materials and methods

2.1. Chemicals

Sm was provided by Sigma Aldrich (St. Louis, MO, USA). Compritol and polyoxyethylene sorbitan monooleate (Tween 80) were obtained from Merck Schuchardt (Darmstadt, Germany). Tumor necrosis factor-alpha (human recombinant *E. coli*) and D-galactosamine were supplied by Gattefosse (Saint-Priest Cedex, France), Duksan Chemical Company (Ansan, South Korea), Calbio Chem and Acros Organics, respectively.

2.2. Animals

In this study, 10-week-old BALB/c male mice (n = 35) weighing 20–25 g were used. The mice were kept under controlled laboratory conditions and were sustained with pellets and tap water. The animals were randomly categorized into 5 groups, each containing 7 mice. They were kept in daylight and dark for 12 h at 22 ± 2 °C and 45%–50% humidity in automated controlled rooms. All procedures concerning the animals in this study were approved by the Anadolu University Animal Welfare Committee.

2.3. Experimental design

The mice were placed in five groups, each containing seven. Group 1 received 0.5 mL of saline i.p. Group 2 received only D-GalN/TNF-α, i.p; group 3 received Sm 4 h after being given i.p. D-GalN/TNF-α; group 4 received Sm-free SLN 4 h after being given D-GalN/TNF-α, i.p; and group 5 received Sm-SLNs 4 h after being given D-GalN/TNF-α, i.p. Groups 1 and 2 were sacrificed 4 h after the injection procedure (Gezginci-Oktayoglu et al., 2008), while the others were sacrificed 24 h after the final injections (Muriel and Mourelle, 1990). TNF-α was administered to each of the mice once it had been dissolved in distilled water in doses of 15 µg/kg. However, Sm was solved in harmless doses of dimethyl sulfoxide (DMSO), and then water was added to this mixture before it was injected to the experimental animals. D-GalN was administrated in doses of 700 mg/kg after being dissolved in distilled water. Finally, 100 mg/kg Sm, which had been dissolved in distilled water, was injected into the mice along with 100 mg/kg Sm-free SLN and Sm-loaded SLN, which had been prepared in solution form (Table 1).

2.4. Biochemical assays

Serums were obtained from the blood samples of the mice used in the experiment for 10 min at 3000 rpm. The samples were then analyzed to determine serum glutathione (GSH) blood urea nitrogen (BUN), creatinine (Cre), and plasma malondialdehyde (MDA) with the help of an automated biochemical auto-analyzer (HITACHI-917).

2.5. Preparation of Sm-loaded SLNs

SLNs were prepared with the hot homogenization technique, as reported by Muller et al. (2000). Lipid matrix was melted at about 80 °C, and a certain amount of Sm was then added. After distillation, water with surfactants (Tween 80) was heated until the same temperature was achieved. The hot lipid phase was poured over the hot water–surfactants solution using an Ultra-Turrax at 20,500 rpm (T25, Janke & Kunkel IKA, Germany), and this was cooled at room temperature so that solid lipid nanosuspension could be obtained.

2.6. Characterization of SLNs

The average diameters (particle size) and polydispersity index (PI) of SLNs were determined with the aid of photon correlation spectroscopy (PCS) using a Nano Zetasizer (ZS, Malvern, UK) at a fixed angle of 90° and at a temperature of 25 °C. The PI is a measure of the size distribution of a nanoparticle population. Before colloidal dispersion

Table 1. Names of the experimental groups, chemicals administered, and doses.

Group name	Hours		
	0	4	28
Group 1 (control)	Saline (0.5 mL)	Sacrificed	
Group 2	700 mg/kg D-GalN /15 µg/kg TNF-α	Sacrificed	
Group 3	700 mg/kg D-GalN /15 µg/kg TNF-α	100 mg/kg Sm	Sacrificed
Group 4	700 mg/kg D-GalN /15 µg/kg TNF-α	100 mg/kg Sm-free SLN	Sacrificed
Group 5	700 mg/kg D-GalN /15 µg/kg TNF-α	100 mg/kg Sm-loaded SLN	Sacrificed

stability could be assessed, the zeta potential was measured with a Nano Zetasizer at 25 °C. SLNs were then suspended in distilled water (pH 7). All the samples were analyzed in triplicate. Sm-loaded SLNs were also characterized by transmission electron microscopy (TEM) (TEM FEI Tecnai BioTWIN). The SLNs were spread over a Cu grid and then stained with uranyl acetate for observation by TEM. The zeta potential of the particles was determined by Malvern Zetasizer Nano ZS (Malvern Instruments). In order to determine Stern potential, some measurements were performed in bidistilled water adjusted with a 0.9% (w/v) sodium chloride solution to a conductivity of 50 µS/cm.

2.7. Histopathological investigations

The kidney tissues harvested from the mice were cut into small pieces and fixed in Bouin's solution. Following dehydration in an ascending ethanol series (70%, 90%, 96%, 100%), the tissue samples were cleared in xylene, embedded in paraffin, and sliced in 5–6 m sections, which were then stained with hematoxylin–eosin (H-E) and Masson's trichrome (Masson), thus exposing collagen.

2.8. Statistical analysis

SPSS 12.0 for Windows was used to assess the data obtained in this study. The numerical value (P) for the difference was significant at $P < 0.05$.

3. Results

3.1. Particle size and zeta potential

To date, SLNs have been prepared with diverse methods that play a crucial part in the particle size, shape, controlled drug release, entrapment efficacy, and stability of SLNs (Koppel, 1972). Particle size is one of the most significant physical properties of colloidal carrier systems when determining physical stability and activity of SLNs (Numanoğlu and Tarımcı, 2006). In the present study, Sm particle sizes changed from 600 nm to nearly 5 µm, while Sm-loaded SLN particle sizes varied between 165 and 200 nm (Table 2). Tween 80 also maintained the stability of SLN (Demirel et al., 2001). Furthermore, SEM images showed that particle size of SLN formulations was smaller than those of Sm, and that Sm had a cubic shape while SLN formulations were circular (Figure 1). Based on these results, it was concluded that SLN formulation reduced the particle size of Sm, which can be observed in Table 2. Our results are consistent with those in the literature (Takka et al., 2005). Predictions about the storage stability of colloidal dispersion were achieved by determining zeta potential. In this study, all the formulations were negatively charged and changed from −21.2 to −25.9 m V. Sm-loaded SLNs showed relatively good physical stability and dispersion quality (Venkateswarlu and Manjunath, 2004). The zeta potential value could be affected by aggregation and storage conditions. However, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion (Al-Haj and Rasedee, 2009). Zeta potential values of Sm-loaded SLNs are shown in Table 2.

3.2. Histological results

Hematoxylin-eosin–stained samples were scrutinized under light microscopy. The structure of the kidney tissues was normal in the control group (group 1). However, kidney structures of the mice given D-GalN/TNF-α (group 2) and D-GalN/TNF-α/Sm-free SLN (group 4) were severely impaired. Based upon the tissue samples, it was determined that the kidneys of the mice had been damaged because there was extensive tubular damage and cellular infiltration in the interstitial region of the kidney cortex, as well as vascular congestion (Figure 2). The kidney samples of group 3 (Figure 2) and group 5 revealed that the kidneys of the mice in group 5 were better protected than those in group 3. Furthermore, the results of group 5 were remarkably similar to those of the control group (Figure 2).

3.3. Biochemical results

D-GalN/TNF-α (group 2) and D-GalN/TNF-α/Sm-free SLN (group 4) administration increased serum BUN and Cre levels significantly. Sm and Sm-loaded SLN significantly decreased serum BUN and Cre levels after D-GalN/TNF-α administration ($P < 0.05$) (Figures 3 and 4). A comparison of serum GSH and plasma MDA levels in group 2 with those of the control group showed a significant decrease in GSH levels, while plasma MDA levels increased dramatically ($P < 0.001$). In group 3, serum GSH levels increased, but plasma MDA levels decreased

Table 2. The particle size, zeta potential, and polydispersity index of Sm, Sm-loaded SLNs, and Sm-free SLNs. Data are presented as mean values and show standard deviation (n = 3).

Formulations	Particle size (nm)		Polydispersity index (PI)		Zeta potential (mV)	
	Mean (n = 3)	S.D.	Mean (n = 3)	S.D.	Mean (n = 3)	S.D
Sm	5000	1.3024	0.562	0.01	-	-
Sm-free SLN	200	1.2087	0.451	0.03	−25	0.007
Sm-loaded SLN	165	0.5247	0.401	0.02	−26.5	0.005

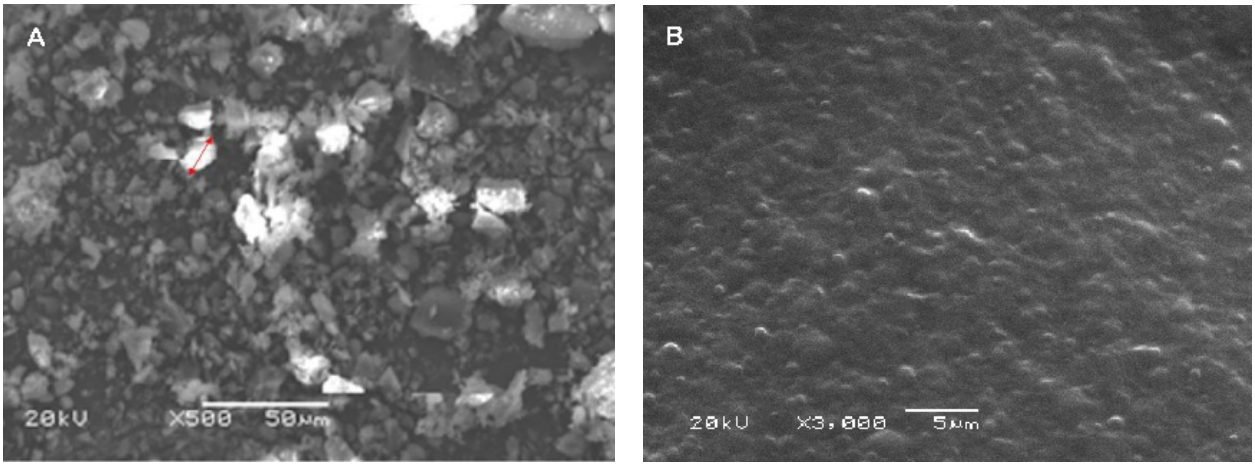

Figure 1. SEM images. Sm (A) and Sm-SLN (B).

when compared to those of group 2 ($P < 0.001$). As for group 4, there was a difference between serum GSH and plasma MDA levels in groups 4 and 2, but this difference did not achieve statistical significance ($P > 0.05$). In group 5, serum GSH levels increased significantly as opposed to plasma MDA levels, which decreased significantly when compared to those in groups 2 and 4 ($P < 0.001$). On the other hand, differences in MDA and GSH levels between groups 3 and 5 had no statistical significance ($P > 0.05$).

4. Discussion

The present study investigates whether Sm-loaded SLN treatment can prevent the development of D-GalN/TNF-α–induced cardio toxicity. Our histological results show the kidney specimens harvested from mice 4 h after they had been given 700 mg/kg D-GalN and 15 μg/kg TNF-α (group 2) and 700 mg/kg D-GalN/15 μg/kg TNF-α/100 mg/kg SLN (group 4). In the same vein, BUN and Cre levels increased (Figures 3 and 4). In addition, plasma MDA levels increased significantly (Table 3) when compared to those of the control group, while serum GSH levels decreased remarkably ($P < 0.001$). Our histological and biochemical findings were consistent with those in the literature.

Anand et al. (2002) showed that treatment with D-GalN results in renal failure, which is manifested by a decrease in renal blood flow, creatinine clearance along with a rise in plasma endothelin-1 (ET-1) concentration, and the upregulation of ET-1 receptor in the renal cortex. This study also suggests that renal failure may develop secondary to severe liver damage. Likewise, GalN administration at a dose of 800 mg/kg for three consecutive days caused enhanced oxidative insult in the kidney tissue, as demonstrated by the increased renal malondialdehyde (MDA) levels and reduction in the activity of endogenous antioxidant enzymes, such as GSH. GalN intoxication also reduced the levels of total thiol and glutathione followed by an increase in oxide glutathione (GSSG) levels. Renal dysfunction due to GalN treatment was also manifested by an increase in serum Cre and urea nitrogen (UN) levels as, compared to the untreated mice (Sinha et al., 2007). Another study reported the D-GalN/TNF-α combination triggers kidney injury, which appears to be secondary to liver injury (Gezginci-Oktayoglu et al., 2008). Gezginci et al. (2008) studied kidney damage caused by a combination of 700 mg/kg D-GalN and 15 μg/kg TNF-α and observed tubular injury, less prominent glomerular injury, shortening at the brush border, and mononuclear cell infiltration 4 h subsequent to D-GalN/TNF-α injection. Moreover, treatment with D-GalN/TNF-α significantly decreased the GSH levels (29.14 ± 4.66 vs. 18.23 ± 1.43). Cre, an indicator of glomerular function of the kidney, and BUN are also employed as indicators in determining the extent of the damage to kidney tissue. Serum creatinine and BUN levels have been reported to increase once the kidney has received damage (Senthilkumar et al., 2006).

The present study also found extensive tubular damage, intensive cell infiltration, and vascular congestion in the kidney cortex, all of which were induced by 700 mg/kg D-GalN and 15 μg/kg TNF-α. Liver tissue damage was reduced in the groups given Sm and Sm-loaded SLN. Likewise, the rise in MDA, BUN, and Cre levels due to kidney damage reversed, and serum GSH levels increased remarkably ($P < 0.001$). However, Sm-loaded SLN has proved to be a more effective alternative than Sm thanks to its superior performance in healing kidney damage (Figure 2). In one study following renal I/R injury, significant tubular dilatation, tubular vacuolization, pelvic inflammation, interstitial inflammation, perirenal adipose infiltration, tubular necrosis, and glomerular necrosis (cortical necrosis) were observed. Nevertheless, histopathological changes due to I/R injury were

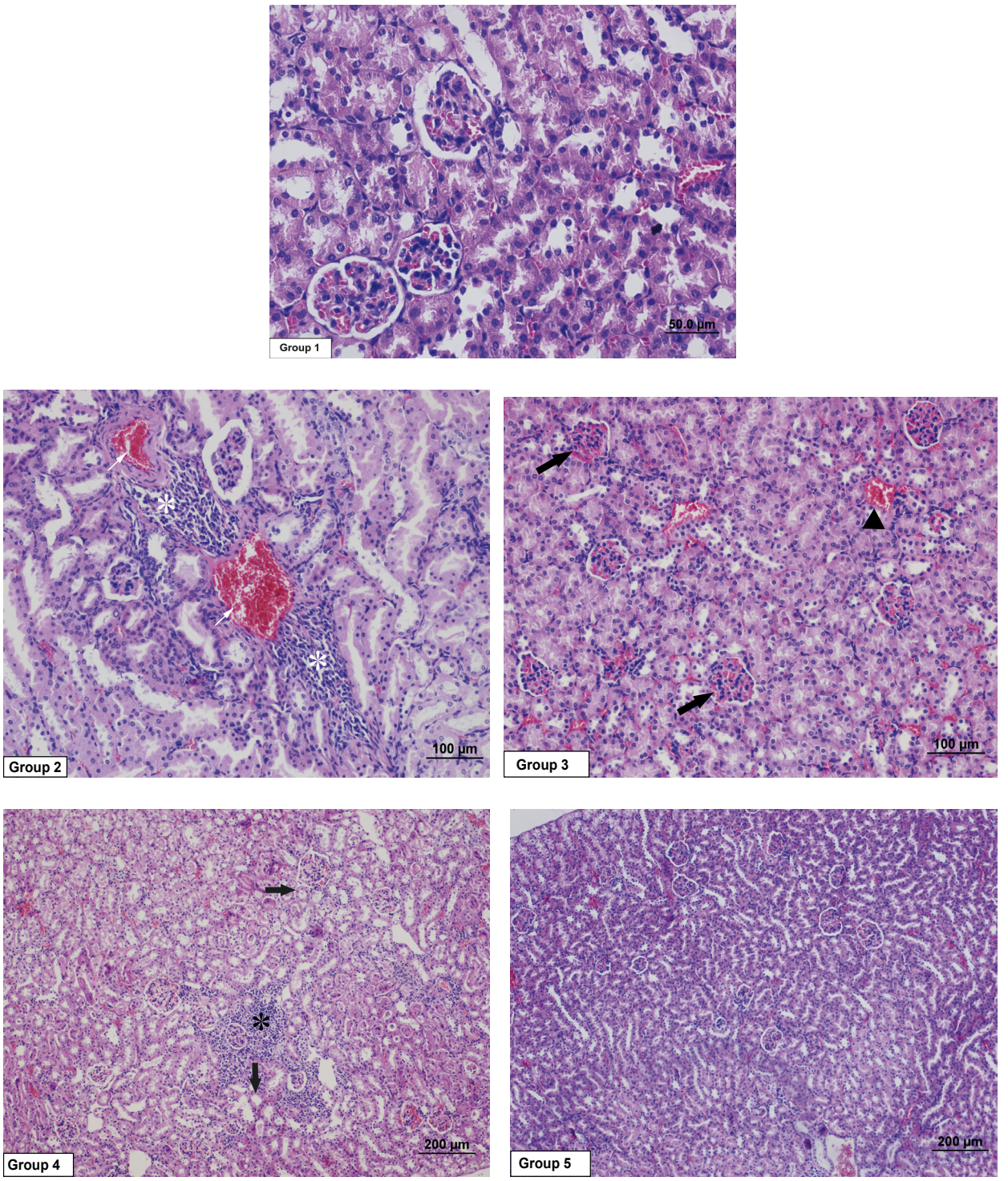

Figure 2. Kidney with normal histological structures (group 1); kidney structures extensively damaged by D-GalN/TNF-α (group 2); [extensive cellular infiltration in the interstitial region of the kidney cortex (*) and vascular congestion (→)]; partial recovery due to Sm in kidney damaged by D-GalN/TNF-α (group 3); [narrowing in the space of Bowman in Malpighian corpuscles of the kidney cortex (→) and some vascular congestion (►)]; kidney with histological structures damaged by D-GalN/TNF-α given prior to Sm-free SLN (group 4) injection; remarkable recovery due to Sm-loaded SLN in the kidney damaged by D-GalN/ TNF-α (group 5).

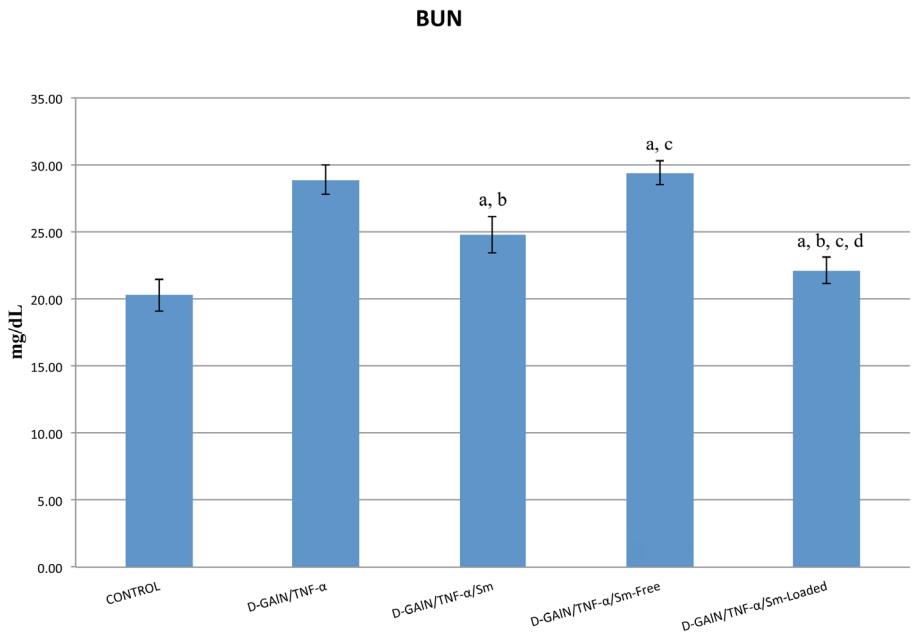

Figure 3. Effect of D-GalN/TNF-α, Sm, Sm-free SLN, and Sm-loaded SLN on the activities of BUN in kidney. All values are the mean ± SD (n = 7). [a]P < 0.001 compared to control, [b]P < 0.001 compared to group 2, [c]P < 0.001 compared to group 3, [d]P < 0.001 compared to group 4.

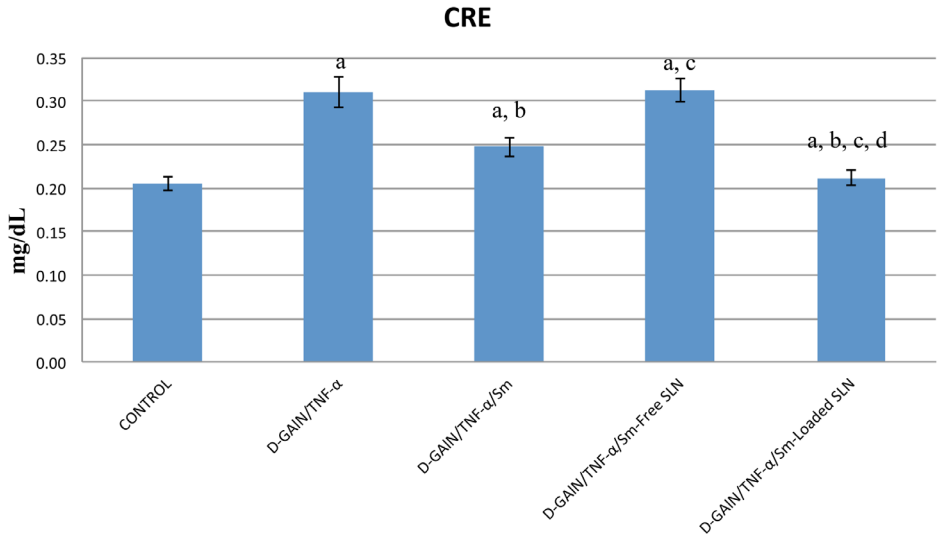

Figure 4. Effect of D-GalN/TNF-α, Sm, Sm-Free SLN, and Sm-loaded SLN on the activities of Cre in kidney. All values are the mean ± SD (n = 7). [a]P < 0.001 compared to control, [b]P < 0.001 compared to group 2, [c]P< 0.001 compared to group 3, [d]P < 0.001 compared to group 4.

prevented, even with a low dose of 100 mg/kg Sm (Senturk et al., 2008). Thus, our findings appear to be in line with those published in the literature. In one study 50 mg/kg Sm was provided to rats in order to cure them of 3 mg/kg cisplatin-induced kidney damage, and Sm prevented BUN and creatinine from increasing (Karimi et al., 2005). In another study, a single dose of adriamycin (10 mg/kg) produced proximal tubular degeneration and cast formation, which characterized nephrotoxicity, and a posttreatment of 50 mg/kg Sm showed protection against ADR-induced nephrotoxicity (El-Shitany et al., 2008). Sm also normalized ADR-induced increases in kidney MDA and restored the depleted GSH contents (Mansour et al., 2006).

Table 3. Serum GSH and plasma MDA levels of blood samples taken from BALB/c mice.

Groups	GSH (nmol/mg protein)	MDA (nmol/mg protein)
Group 1 (control)	31.4 ± 2.15	0.31 ± 0.03
Group 2	5.16 ± 0.33 ***	1.38 ± 0.11***
Group 3	18 ± 0.8 *** ###	0.74 ± 0.06*** ###
Group 4	5.08 ± 0.35 *** ΔΔΔ	1.39 ± 0.11*** ΔΔΔ
Group 5	27.43 ± 3.11**### ΔΔΔ @@@	0.39 ± 0.04### ΔΔΔ @@@

All values are the mean ± SD (n = 7). Statistical significance between means was performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer as a post-ANOVA test ($P < 0.05$). Means with the same letter within the same parameter are not significantly different. **$P < 0.05$ compared to control, ***$P < 0.001$ compared to control, ### $P < 0.001$ compared to group 2, ΔΔΔ $P < 0.001$ compared to group 3, @@@ $P < 0.001$ compared to group 4.

In conclusion, Sm-loaded SLN was found to be more effective in curing kidney damage than Sm, which can be attributed to the slow and regular release of Sm by nanoparticles providing Sm with a rise in bioavailability, which, in turn, improves its therapeutic effects.

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References

Al-Haj N, Rasedee A (2009). Solid lipid nanoparticles preparation and characterization. Int J Pharmacol 1: 90-93.

Anand R, Harry D, Holt S, Milner P, Dashwood M, Goodier D, Jarmulowicz M, Moore K (2002). Endothelin is an important determinant of renal function in a rat model of acute liver and renal failure. Gut 50: 111-117.

Basaga H, Poli G, Tekkaya C, Aras I (1997). Free radical scavenging and anti-oxidative properties of 'silibin' complexes on microsomal lipid peroxidation. Cell Biochem Funct 15: 27-33.

Bertoli D, Segal S (1966). Developmental aspects and some characteristics of mammalian galactose 1 phosphate uridyltransferase. J Biol Chem 241: 4023-4029.

Bourdi M, Masubuchi Y, Reilly TP, Amouzadeh HR, Martin JL, George JW, Shah AG, Pohl LR (2002). Protection against acetaminophen-induced liver injury and lethality by interleukin 10: role of inducible nitric oxide synthase. Hepatology 35: 289-298.

Bradham CA, Plumpe J, Manns MP, Brenner DA, Trautwein C (1998). Mechanism of hepatic toxicity. TNF-induced liver injury. Am J Physiol 275: 387-392.

Cuatrecasas P, Segal S (1965). Mammalian galactokinase: developmental and adaptive characteristics in the rat liver. J Biol Chem 240: 2382-2388.

Demirel M, Yazan Y, Muller RH, Kılıç F, Bozan B (2001). Formulation and in vitro-in vivo evaluation of piribedil solid lipid micro and nanoparticles. J Microencapsul 18: 359-371.

El-Shitany NA, El-Haggar S, El-Desoky K (2008). Silymarin prevents adriamycin-induced cardiotoxicity and nephrotoxicity in rats. Food Chem Toxicol 46: 2422-2428.

Gezginci-Oktayoglu S, Tunali S, Yanardag R, Bolkent S (2008). Effects of Z-FA.FMK on D-galactosamine/tumor necrosis factor-alpha-induced kidney injury and oxidative stress in mice. Mol Cell Biochem 309: 9-20.

Karimi G, Ramezani M, Tahoonian Z (2005). Cisplatin nephrotoxicity and protection by milk thistle extract in rats. Adv Access Publication 26 July 2005 eCAM 2: 383-386.

Koppel DE (1972). Analysis of macromolecular dispersity in intensity correlation spectroscopy: the method of cumulants. J Chem Phys 57: 4814.

Kren V, Walterová D (2005). Silybin and Sm—new effects and applications. Biomed Pap 149: 29-41.

Leist M, Gantner F, Bohlinger I, Germann PG, Tiegs G, Wendel A (1994). Murine hepatocyte apoptosis induced in vitro and in vivo by TNF-alpha requires transcriptional arrest. J Immunol 153: 1778-1788.

Lorenz D, Lucker P, Mennicke W, Wetzelsberger N (1984). Pharmacokinetic studies with silymarin in human serum and bile. Method Find Exp Clin 6: 655-661.

Morio LA, Chiu H, Sprowless KA, Zhou P, Heck DE, Gordon MK, Laskin DL (2001). Distinct roles of tumour necrosis factor-alpha and nitric oxide in acute liver injury induced by carbon tetrachloride in mice. Toxicol Appl Pharm 172: 44-51.

Mansour HH, Hafez HF, Fahmy NM (2006). Silymarin modulates cisplatin induced oxidative stress and hepatotoxicity in rats. J Biochem Mol Biol 39: 656-661.

Mudit V, Santosh KK (2010). Molecular mechanisms of inhibition of photocarcinogenesis by Sm, a phytochemical from milk thistle (*Silybum marianum* L. Gaertn) (review). Int J Oncol 36: 1053-1060.

Muriel P, Mourelle M (1990). Prevention by silymarin of membrane alterations in acute CCl_4 liver damage. J Appl Toxicol 10: 275-279.

Numanoğlu U, Tarımcı N (2006). Characterization of solid lipid nanoparticles (SLNTM) and their pharmaceutical and cosmetic applications. Ankara J Fac Pharm 35: 211-235 (article in Turkish with an abstract in English).

Pritchard DJ, Butler WH (1989). Apoptosis-the mechanism of cell death in dimethylnitrosamine-induced hepatotoxicity. J Pathol 158: 253-260.

Provinciali M, Papalini F, Orlando F, Pierpaoli S, Donnini Paolo Morazzoni A, Riva A, Smorlesi A (2007). Effect of the silybin-phosphatidylcholine complex (IdB 1016) on the development of mammary tumors in HER-2/neu transgenic mice. Cancer Res 67: 2022-2029.

Ring-Larsen H, Palazzo U (1981). Renal failure in fulminant hepatic failure and terminal cirrhosis: a comparison between incidence, types, and prognosis. Gut 22: 585-591.

Sakaguchi S, Yokota K (1995). Role of Ca^{2+} on endotoxin-sensitivity by galactosamine challenge: lipid peroxide formation and hepatotoxicity in zymosan primed mice. J Pharmacol Toxicol 77: 81-86.

Schulz H, Schurer M, Krumbiegel G, Wachter W, Weyhenmeyer R, Seidel G (1995). The solubility and bioequivalence of silymarin preparations. Arznei-Forschung 45: 61-64.

Senthilkumar S, Devaki T, Manohar BM, Babu MS (2006). Effect of squalene on cyclophosphamide-induced toxicity. Clin Chim Acta 364: 335-342.

Senturk H, Kabay S, Bayramoglu B, Ozden H, Yaylak F, Yucel M, Gurlek E, Kutlu A (2008). Silymarin attenuates the renal ischemia/reperfusion injury-induced morphological changes in the rat kidney. World J of Urol 26: 401-407.

Shi J, Aisaki K, Ikawa Y, Wake K (1998). Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. Am J Pathol 153: 515-25.

Sinha M, Manna P, Sil CP (2007). Amelioration of galactosamine-induced nephrotoxicity by a protein isolated from the leaves of the herb, *Cajanus indicus* L. BMC Complem Altern M 7: 11.

Sonnenbichler J, Scalera F, Sonnenbichler I, Weyhenmeyer R (1999). Stimulatory effects of silibinin and silicristin from the milk thistle *Silybum marianum* on kidney cells. J Pharmacol Exp Ther 290: 1375-1383.

Sracyn M, Kozlowski W, Wanikowicz Z (2004). Description of an experimental model of hepatorenal syndrome in Wistar rats. Pol Merkur Lekarski 17: 19-122.

Takka S, Tursilli R, Bianchi A, Scalia S (2005). Assay of common sunscreen agents in suncare products by high performance liquid chromatography on a cyanopropyl bonded silica column. J Pharmaceut Biomed 38: 250-255.

Tapalaga D, Tiegs G, Angermuller S (2002). NF*R*B and caspase-3 activity in apoptotic hepatocytes of galactosamine-sensitized mice treated with TNF-a. J Histochem Cytochem 50: 1599-1609.

Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey III TJ, Zentelia A, Albert JD, Shires GT, Cerami A (1986). Shock and tissue injury induced by recombinant human cachectin. Science 234: 470-474.

Venkateswarlu V, Manjunath K (2004). Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release 95: 627-638.