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Sofosbuvir's hepatoprotective efficacy in rats is enhanced by encapsulating in taurocholate-stabilized galactose-anchored bilosomes

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Abstract

Background In conjunction with other antiviral medicines, sofosbuvir (SOF) is an essential therapy for chronic hepatitis C. There is some debate over its influence on hepatic fibrosis. The use of nanotechnology in treatment has gained popularity, with the goal of delivering therapeutic substances to the liver to increase efficacy and decrease adverse effects. The aim of this study was to demonstrate the protective effect of sofosbuvir and the efficacy of incorporating nanoparticle galactosylated taurocholate bilosomal formula to SOF on thioacetamide-induced liver fibrosis.

Methods Rats were divided into 7 groups: normal control, SOF, SOF encapsulated in galactosylated taurocholate bilosomal formula (nano-SOF), galactosylated taurocholate bilosomal formula (nanoparticle), thioacetamide (TAA), TAA-SOF and TAA-nano-SOF. Liver fibrosis was induced by TAA (200 mg/kg) intraperitoneal injection twice per week for 8 weeks. SOF, nanoparticle and nano-SOF were given (40 mg/Kg/day) orally from day one of the study. Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and tissue transforming growth factor beta (TGF-β) were assessed. Also, histopathological assessment of hepatic tissue was done.

Results Administration of SOF and TAA to normal rats resulted in significant increase in serum AST, ALT, ALP and tissue TGF-β_1 levels with variable degree of liver fibrosis. Additionally, rats in TAA group that received SOF therapy did not exhibit improved liver functions, TGF-β_1 level and liver fibrosis score. However, administering nano-sofosbuvir prophylactically to TAA-treated rats resulted in a considerable improvement in liver function tests, TGF-1 levels, with liver fibrosis score regression.

Conclusion In contrast to free sofosbuvir, SOF encapsulated in galactosylated taurocholate bilosomal formula (nano-SOF) displayed hepatoprotective effects in rat with thioacetamide-induced hepatic fibrosis. These findings strongly support the concept that galactoylatedbilosomes are promising nanocarrier for the targeted delivery of sofosbuvir to the liver.

Keywords Bilosomal formulation, Galactose, Taurocholate, Hepatic fibrosis, Sofosbuvir, Thioacetamide

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Background

Chronic liver illnesses are frequently linked with hepatic fibrosis, a potentially fatal consequence that has a significant financial and medical cost [1]. Pathophysiology of hepatic fibrosis is primarily driven by the stimulation, proliferation and transformation of hepatic stellate cells (HSCs) into myofibroblasts due to variable etiology [2].

Sofosbuvir (SOF) was authorized for the treatment of chronic hepatitis C in combination with other antiviral providing 90% cure rates [3]. Thus, it has been used extensively worldwide as a cornerstone treatment for chronic hepatitis C infection [4]. Sofosbuvir is classified as a class III medication due to its poor permeability and high solubility in the biopharmaceutics categorization system [5]. Additionally, SOF has challenges related to its poor bioavailability and limited liver targeting. When administered orally, 36.4% of the dose is absorbed into the portal circulation, and 74% is extracted hepatically, making 26.94% of the dose available in the liver [6]. Moreover, sofosbuvir has a 127-L volume of distribution across the body, which results in a wide range of adverse effects [7]. Sofosbuvir has been the focus of much research, and while there is disagreement concerning its impact on the liver, both hepatoprotective and hepatotoxic effects have been shown [8].

Nanotechnology allows for the precise delivery of therapeutic substances into the tissue, and therapy using this technology has garnered more interest recently [9]. The most widely used and researched nanovesicles are liposomes. Bilosomes are modified liposomes that have bile acids or bile salts added [10]. The encapsulation of SOF in bilosomes protects the drug from degradation in the digestive system, and its lipid-based, provides improving in the solubility and permeability of SOF allowing it to be absorbed more effectively and transported to the liver [11]. Moreover, the incorporation of taurocholate—a bile salt—into the bilosomal formulation enhances the drug's hepatic targeting. Bile salts like taurocholate interact with specific receptors on hepatocytes to facilitate the uptake of the bilosomes directly into liver cells. This mechanism ensures that SOF is delivered efficiently to its target site, reducing off-target effects and improving the therapeutic efficacy of the drug. [12]. Additionally, the small particle size of the bilosomes (around 140 nm) further enhances the drug's cellular uptake by facilitating its passage through the liver's cell membranes. Also, the presence of dual surfactant agents (sodium taurocholate (STC) and span 60 (S60)) might fluidize the membrane lipid components, thereby promoting drug permeation [13].

Moreover, galactose serves as a vector for the active targeting of the medication contained in the galactosylated nanovesicular carrier as it has receptors on the hepatocytes [14]. The created bilosomes used two vectors: Galactose and bile salts to maximize the bioavailability of SOF while ensuring its specific targeting to hepatocytes, thereby addressing both the bioavailability challenge and the need for targeted liver delivery in the treatment of liver diseases like chronic hepatitis C. Also, this formulation minimizes systemic exposure and the associated side effects of SOF [15]. So**,** to increase liver targetability of sofosbuvir, its nanocarrier formula was synthesized using galactosylated taurocholate bilosomal formula in this study.

The aim of this work was to evaluate the protective effect of sofosbuvir and that of SOF encapsulated in galactosylated taurocholate bilosomal formula, on a non-HCV model of thioacetamide-induced liver fibrosis in rats.

Methods

Materials

Sofosbuvir powder was obtained as a kind gift from Marcyrl Pharmaceutical Industries. Before being used, the powder has been freshly prepared by dissolving it in distilled water.

L-a-Phosphatidylcholine (PC), span 60 (S60), sodium taurocholate (STC) and galactose were purchased from Sigma-Aldrich, St. Louis, MO, USA. Thioacetamide powder (Sigma-Aldrich Company) was dissolved in normal saline. The residual solvents and chemicals were analytical grade and used without additional purification. Commercial kits to measure the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were obtained from Bio diagnostic Co. (Dokki, Giza, Egypt). Kits for assessment of tissue transforming growth factor-β1 (TGF-β1) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Animals

Seventy healthy adult male Wister rats weighing between 200 and 250 g were included in this study. Rats were kept in standard laboratory settings with a 12-h light and dark cycle and a temperature of 25 ± 2 °C. The animals were allowed to water ad libitum and had free access to a standard chow diet. The current study was approved by the Institutional Animal Care and Use Committee, Cairo University (IACUC) (approval No: CU/ III F9119).

Induction of hepatic cirrhosis

To induce hepatic cirrhosis, thioacetamide (TAA) (200 mg/kg, dissolved in normal saline) was injected intraperitoneal to rats twice per week for 8 weeks [16].

Preparation and characterization of SOF encapsulated in galactose-anchored bilosomal formulas The preparation was done using a validated method. Briefly, through a one pot acid condensation reaction between amide and aldehyde (STC and D-galactose), galactosylated taurocholate was produced [17]. A reaction between 1.2 g of STC and 1 g of D-galactose was allowed to occur in a Dean–Stark water trap for 7 h at 110 °C in 70 ml of xylene with pH 4 adjusted with HCl. A precipitate that was dark brown in color had developed after 7 h. It was rinsed three times using distilled water and ethanol saved for more reactions and analysis. Using an FTIR spectrophotometer **(Nicolet iS10, Thermo Fisher Scientific, Waltham, MA, USA)**, the Fourier transform infrared (FTIR) spectra of STC and galactosylated taurocholate were recorded on KBr pellets. STC and galactosylated taurocholate were dissolved in DMSO-d6 to perform 1-h nuclear magnetic resonance on a 400 MHz spectrometer **(Bruker LLC, Billerica, MA, USA)** [15]. Using the thin-film hydration method, bilosomes were created [18]. SOF (100 mg), STC, PC and S60 were precisely weighed, dissolved in a 10 mL methanol–methylene chloride combination at a 1:3 v/v ratio and then put into a 250-mL round-bottomed flask. The organic solvent mixture was evaporated under vacuum using a rotary evaporator **(Rotavapor, HeidolphVV 2000, Burladingen, Germany)** that rotated at 80 rpm for 30 min at 50 °C. 10 mL of double-distilled water was used to hydrate the wall-assembled thin film at normal pressure. In order to prevent aggregation, the produced bilosomes were lastly sonicated for three minutes in an ultrasonic bath **(model SH 150-41, PCI Analytics Pvt. Ltd, Mumbai, India)** [19]**.**

The full preparation of the galactosylated taurocholate, along with the formulation of the bilosomes, characterization and statistical analysis, has already been published by Joseph Nagibe et al. [15]. The characterization of the prepared SOF encapsulated in galactose-anchored bilosomal formulas, in terms of polydispersity index, analysis of vesicular size and zeta potential, and also the determination of the encapsulation efficiency and the drug loading of the prepared sofosbuvir bilosomes were done, and all the results were in line with the results of previous published study by Joseph Nagibe et al. [15]

Experimental design

Rats were randomly allocated into seven groups of ten rats each following a week-long period of acclimation: **Group I**, normal control: rats received distilled water orally for 8 weeks; **Group II**, SOF group: rats received sofosbuvir (SOF) (40 mg/Kg/day, oral) [20]; **Group III**, nano-SOF group: rats received SOF encapsulated in galactose-anchored bilosomal formulas (40 mg/Kg/day, oral) (equivalent to 40 mg/kg body weight of SOF) [21]; **Group IV**, nanoparticle group: rats received galactosylated taurocholate bilosomal formula (40 mg/Kg/day, oral) (equivalent to 40 mg/kg body weight of SOF) [21]; **Group V**, liver fibrosis group: liver fibrosis was induced in rats by thioacetamide (TAA) (200 mg/Kg, intraperitoneal, twice weekly) for 8 weeks [16]; **Group VI**, TAA + sofosbuvir group: rats received sofosbuvir (40 mg/Kg/day, oral) at the same time of TAA injection; and **Group VII**, TAA + nano-SOF group: rats received SOF encapsulated in galactose-anchored bilosomal formulas orally at the same time of TAA injection. The duration of the study was 8 weeks.

Measurements

Biochemical measurements

Liver function tests

Serum levels of ALT, AST and ALP: After 8 weeks from the start of the experiment, venous samples were collected from the rats' tail veins. The collected blood samples were incubated at 37 °C until blood clotted and then centrifuged at 3000 rpm for 10 min at 4 °C to separate the sera. Serum was separated and stored at − 20 °C until analyzed spectrophotometric using serum **ALT**, **AST** and **ALP** kits **(Biodiagnostic Co.)**

Biomarker of hepatic fibrosis

Homogenate of the liver was prepared for estimation of tissue TGF-β_1. The liver was removed and immediately rinsed with cold phosphate saline (PBS). On a glass plate that was ice-cold, more dissection was made. To achieve 20% w/v homogeneity, the homogenates were made in a Teflon–glass tissue mixer using chilled 50 mM PBS (pH 7.4) [22]. After that, it was centrifuged separately in a chilled centrifuge for 15 min at 3000 rpm. The supernatant was used to determine **TGF-β_1** using rat-specific ELISA reagent kits.

Histopathological assessment of hepatic fibrosis

At the end of the experiment, rats were killed, liver tissue sample was dissected from each rat, and the specimens were kept in 10% formol saline, then cut off, cleaned and dehydrated in ascending grades of alcohol. After being cleaned in xylene, the dehydrated specimens were embedded in paraffin blocks and sectioned at 4–6 µm thick. For histological grading, the resulting tissue slices were deparaffinized with xylol, stained with hematoxylin and eosin (H&E), and then stained with Mason's trichrome stain. Eight microscopic fields per section were examined using the electric light microscope (Olympus

BX50, Japan) under high-power magnification (×400). Correlation between three simple staging systems for liver cirrhosis was done [23].

Statistical analysis

The statistical software for the social sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA) was used to code and input the data. For quantitative variables, the mean and standard deviation were used to summarize the data; for categorical variables, the frequencies (number of instances) and relative frequencies (percentages) were used. Analysis of variance (ANOVA) with multiple comparisons post hoc test was used to compare numerical data across groups [24]. We used the Chi-square (χ^2) test to compare nominal data. Fisher's exact test was used instead when the expected frequency was less than 5 [25]. *P* values were considered statistically significant if they were less than 0.05.

Results

Effect of sofosbuvir, nano-SOF and nanoparticle formula on liver function

Administration of sofosbuvir (SOF) (group II) or thioacetamide (TAA) (group V) to normal rats resulted in significant increase in mean serum levels of ALT, AST and ALP compared to normal control group I. Also, treatment with SOF in group VI (TAA–SOF-treated group) significantly increases the mean serum (AST, ALT and ALP) level compared to TAA control group.

There was significant reduction in mean serum (AST, ALT and ALP) level in TAA–nano-SOF-treated group VII, compared to TAA-treated group V, and TAA–sofosbuvir-treated group VI (Table 1 and Fig. 1.)

Effect of sofosbuvir, nano-SOF and nanoparticle formula on tissue TGF-β_1 level

A significant increase in mean tissue TGF-β_1 level was observed in all treated groups (II–VII), which was more pronounced with TAA (group V) and TAA + SOF (group VI) compared to normal control group I. There was significant reduction in mean tissue TGF-β_1 level in TAA–nano-SOF-treated group VII compared to TAA control group V and TAA–sofosbuvir-treated group VI (Table 1 and Fig. 2).

So, prophylactic treatment of fibrotic rats with SOF did not ameliorate the liver function tests measured nor the marker of fibrosis. However, there was a significant reduction in liver function tests and in liver fibrosis parameter in rats treated with TAA plus nano-SOF.

Liver histopathology

Liver cirrhosis was assessed according to correlation between three simple staging system IASL, Batts–Ludwig and METAVIR (Fig. 3) [23].

Liver tissue sections from rats receiving saline (normal control group I), nanoparticle (group III) and nano-SOF (group IV) showed normal histological structure of hepatic lobules and organization of hepatic cords with prominent central hepatic vein which was observed in 100% of rats [no fibrosis, stage 0—F0] (Figs. 4 and 5a and b).

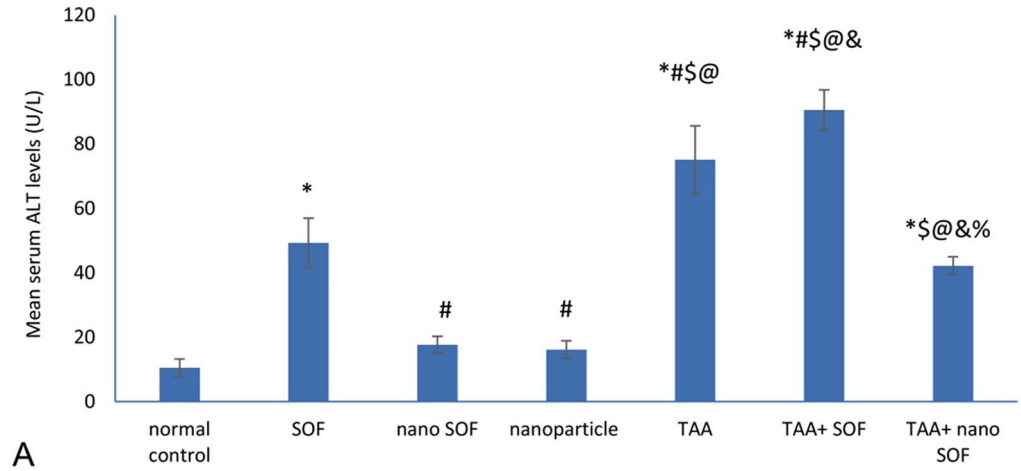
However, SOF administration to normal rats (group II) revealed ballooning degeneration of hepatocytes, intracellular fat droplets and nuclear pyknosis. Multiple focal necrotic areas scatter all hepatic parenchyma, and narrowing of hepatic sinusoids, hyperplasia of Kupffer cells, marked dilatation of portal vein, hyperplasia of bile duct and thickening and hyalinosis of hepatic artery were also seen. Mason's trichrome stain was revealed [moderate periportal fibrosis stage 2—F2] (Figs. 4 and 6a and b).

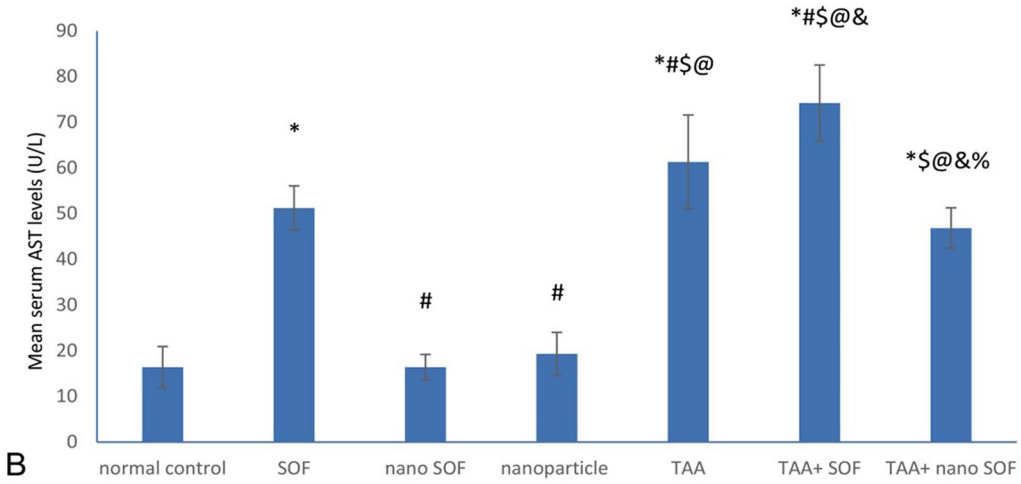
Table 1 Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and tissue transforming growth factor-β1 (TGF-β1) levels (mean ± SD) in different groups studied ($n = 10$)

Groups	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)	Tissue TGF-β1 (pg/ml)
Group I (normal control)	16.4 ± 4.5	10.5 ± 2.72	124 ± 17.66	47.82 ± 3.81
Group II (sofosbuvir (SOF))	51.2 ± 4.85*	49.3 ± 7.66*	278.6 ± 18.33*	184.82 ± 39.37*
Group III (nano-sofosbuvir (nano-SOF))	19.3 ± 4.69#	16.2 ± 2.66#	153.7 ± 26.87#	91.67 ± 6.15*#
Group IV (galactosylated taurocholate bilosomal formula (nanoparticle))	16.4 ± 2.76#	17.7 ± 2.63#	164.4 ± 51.23#	100.02 ± 6.39*#
Group V (thioacetamide (TAA))	61.3 ± 10.26*#$@	75.1 ± 10.54*#$@	365.3 ± 51.07*#$@	306.28 ± 15.63*#$@
Group VI (TAA + SOF)	74.2 ± 8.35*#$@&	90.5 ± 6.28*#$@&	404.5 ± 54.31*#$@	390.47 ± 32.94*#$@&
Group VII (TAA + nano-SOF)	46.8 ± 4.42*$@&%	42.2 ± 2.74*$@&%	225.7 ± 11.77*#$@&%	171.88 ± 14.52*$@&%

Significant *p* value < 0.05

*compared to group I, # compared to group II, $compared to group III, @compared to group IV, &compared to group V and %compared to group VI





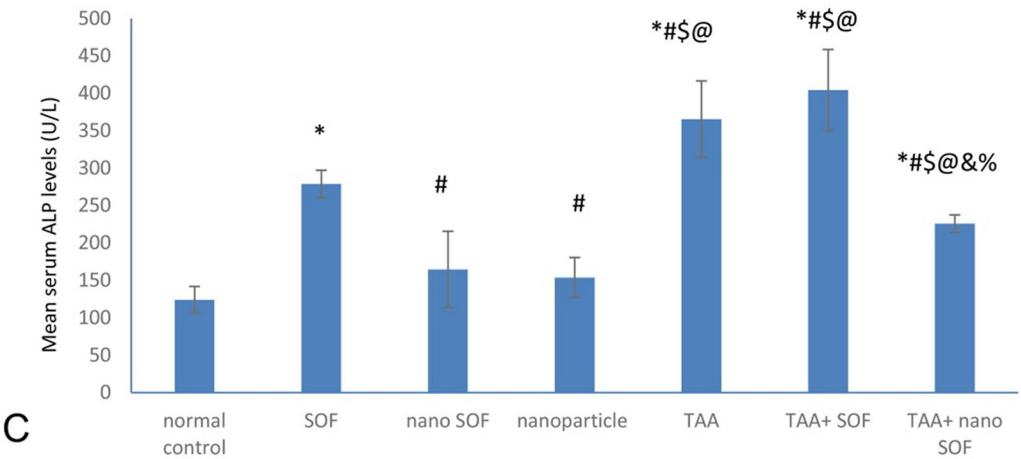


Fig. 1 **A** Serum alanine aminotransferase (ALT) level, **B** serum aspartate aminotransferase (AST) level and **C** serum alkaline phosphatase (ALP) level (mean ± SD) in different groups studied ($n = 10$). Significant p value < 0.05. *compared to group I (normal control), $^{\#}$compared to group II (sofosbuvir (SOF)), $^{\$}$: compared to group III (nano-sofosbuvir (nano-SOF)), $^{@}$compared to group IV (galactosylated taurocholate bilosomal formula (nanoparticle)),$^{\&}$compared to group V (thioacetamide (TAA)) and $^{\%}$compared to group VI (TAA + SOF)

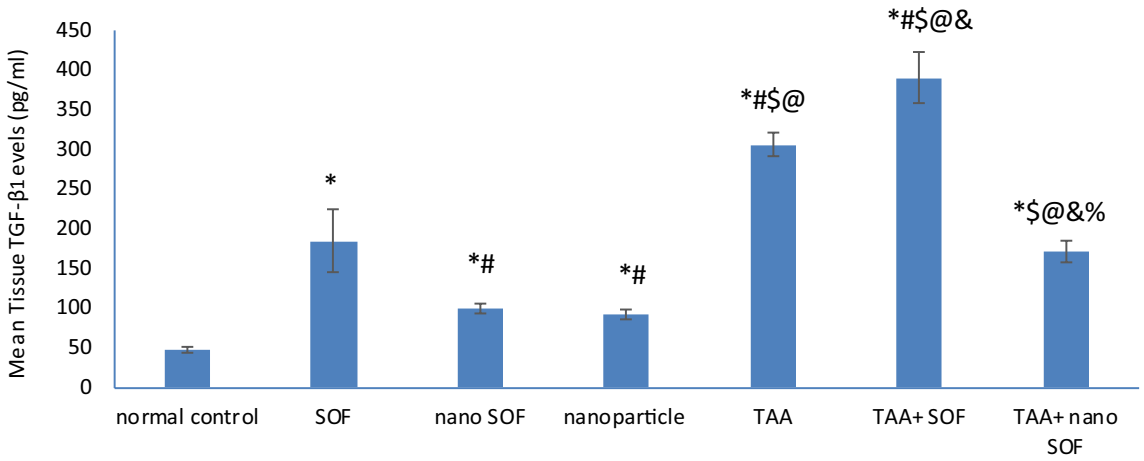


Fig. 2 Tissue transforming growth factor-β1 (TGF-β1) levels (mean ± SD) indifferent groups studied ($n = 10$). Significant p value < 0.05. [*]compared to group I (normal control), [#]compared to group II (sofosbuvir (SOF)), [$]: compared to group III (nano-sofosbuvir (nano-SOF)),[@]compared to group IV (galactosylated taurocholate bilosomal formula (nanoparticle)),[&]compared to group V (thioacetamide (TAA)) and [%]compared to group VI (TAA + SOF)

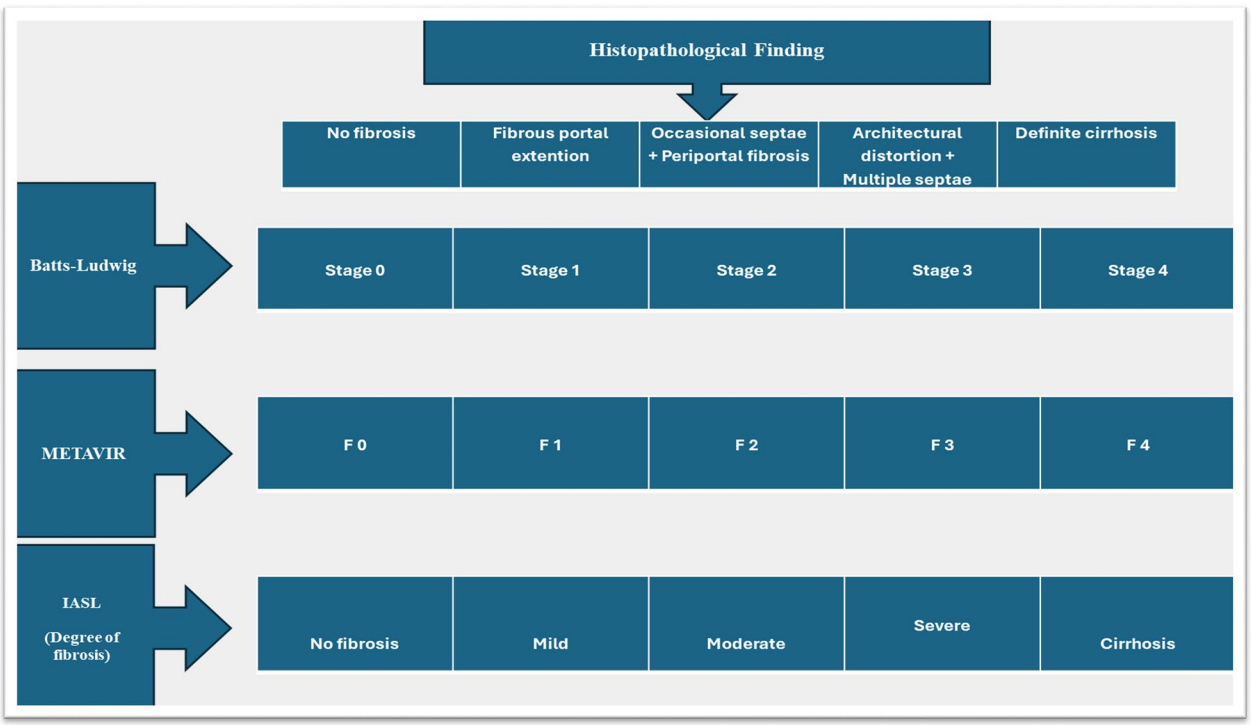


Fig. 3 Correlation between three simple staging systems for liver cirrhosis [23]

Thioacetamide given to rats twice weekly for 8 weeks (group V) produced multinodular liver characterized by varied size nodules with the absence or eccentric central vein. Nuclear pyknosis, multiple focal necrotic areas scatter all hepatic parenchyma, and narrowing of hepatic sinusoids and hyperplasia of Kupffer cells were noticed. The portal tirade showed dense bridging fibrous connective tissue proliferation infiltrated by mononuclear cells mainly macrophages and lymphocytes and hyperplasia of bile duct in which newly formed bile ductulus were seen. Server dilatation of portal vein in all hepatic lobules was detected. Mason's

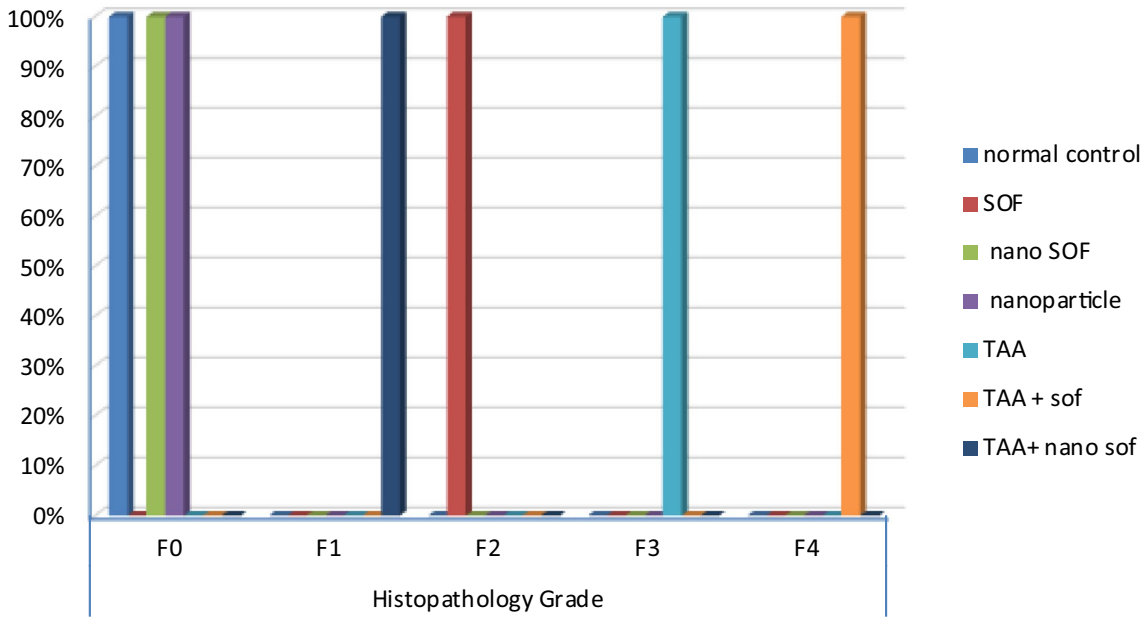


Fig. 4 A grading of histological hepatic lesion according to the number of animals (%) in experimental groups using METAVIR score system

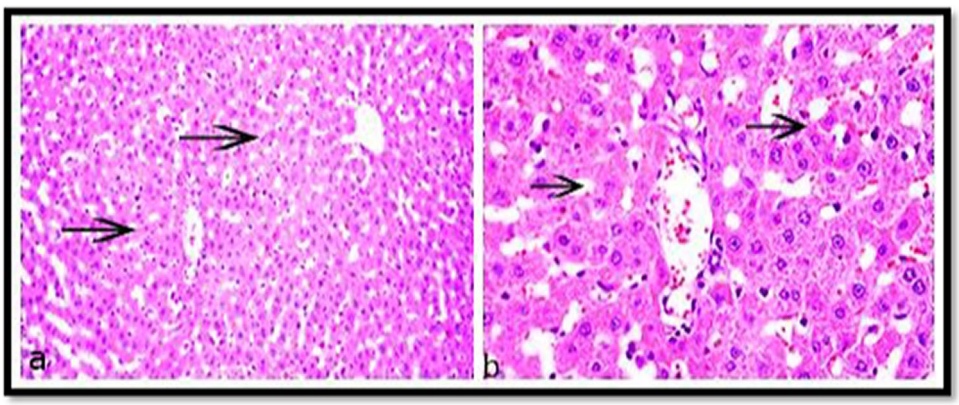


Fig. 5 Liver tissue section from normal control (group I), nanoparticle (group III) and nano-SOF (group IV), showing: **a** normal histological structure of hepatic lobules and organized hepatic cords **arrow** (× 200); **b** polygonal hepatic cells were joined to one another in anastomosing plates **arrow** (H&Ex400)

trichrome-stained tissue section revealed massive fibrous connective tissue proliferation which divided hepatic lobules into several regenerated nodules of varied size and shape [Cirrhosis stage 4—F4] was seen (Figs. 4 and 7a–f).

Prophylactic administration of sofosbuvir (SOF) to rats at the same time with TAA caused similar pathological picture of untreated group V which was observed in 100% of rats [cirrhosis—stage 4—F4] (Figs. 4 and 8a–f).

However, liver sections from cirrhotic model (TAA model) receiving nano-SOF prophylactic (group VII) exhibited considerable reduction in liver fibrosis which was perceived in all rats. The hepatic lobules showed disorganization of hepatic cords with degeneration of hepatocytes in the form of swelling and intracellular fat droplets. Delict fibrous connective tissue in portal tirade, apoptosis of hepatocytes appeared as deeply eosinophilic bodies with few numbers of mononuclear cells infiltration and hyperplasia of Kupffer cells were seen. Mason's trichrome-stained tissue section showed more improvement than the previous group which appeared as delicate fibrous connective tissue proliferation around

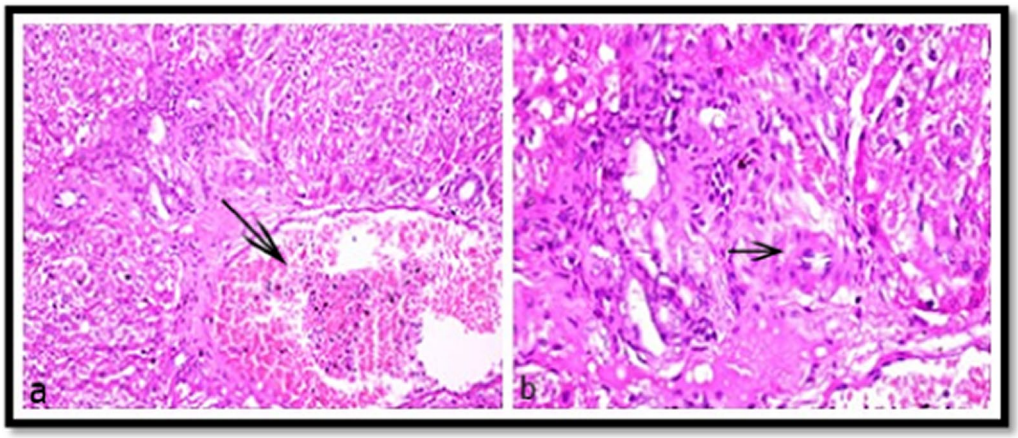


Fig. 6 Liver tissue section from SOF group (group II) showing: a Marked dilatation of portal vein **arrow** (×200); **b** hyalinosis of hepatic artery **arrow** (H&Ex400)

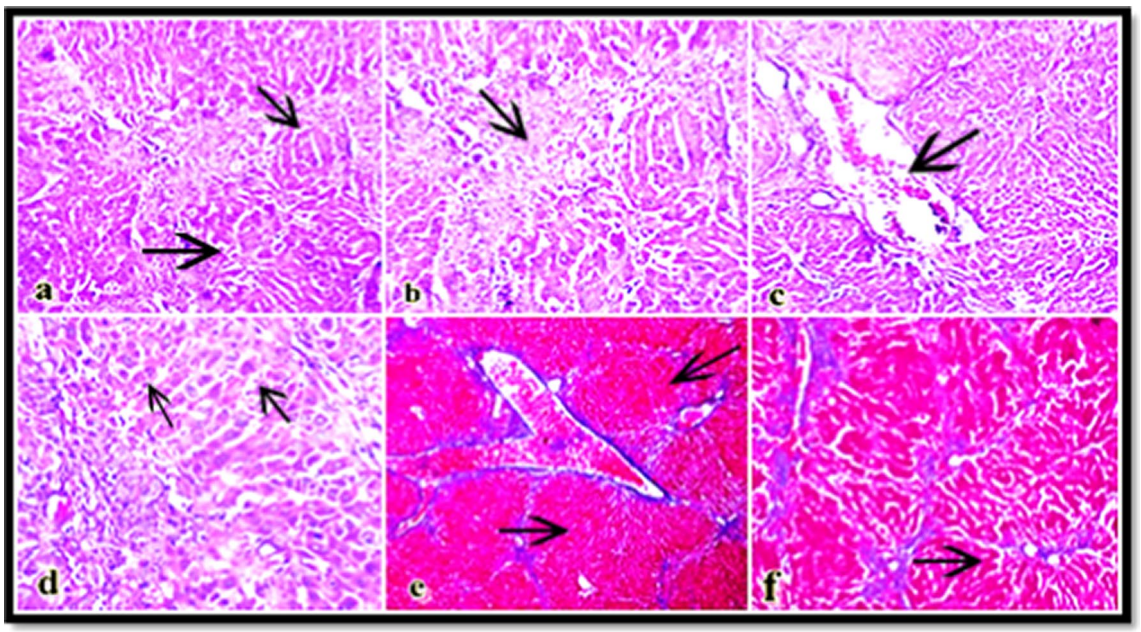


Fig. 7 Liver tissue section from TAA model group V showing: a multinodular liver characterized by varied size nodules **arrow** (×100), **b** dense bridging fibrous connective tissue proliferation infiltrated by mononuclear cells **arrow** (×200), **c** server dilatation of portal vein (×100), **d** nuclear pyknosis and apoptosis of hepatocytes **arrow** (H&Ex400), **e** multiple nodular lesions with severally congested portal vein **arrow** (MTCx100), **f** regenerated nodules without central veins **arrow** (MTCx200)

portal triads with normal architecture of hepatic lobe score [mild periportal fibrosis stage 1—F1] (Figs. 4 and 9a–f).

Discussion

Sofosbuvir is one of the direct acting antivirals (DAAs), a non-structural protein 5B (NS5B) inhibitor, routinely used against all genotypes of hepatitis C virus (HCV). Sofosbuvir have been subjected to intense investigation to demonstrate their hepatic effects, with a controversy, whereas both hepatoprotective and hepatotoxic effects have been reported [8, 26].

One of the most efficient and novel therapeutic agents are nanoparticles (NPs), as their potential ability to target the therapeutic agent to the diseased organ with a concomitant decrease of side effects and enhance its bioavailability.

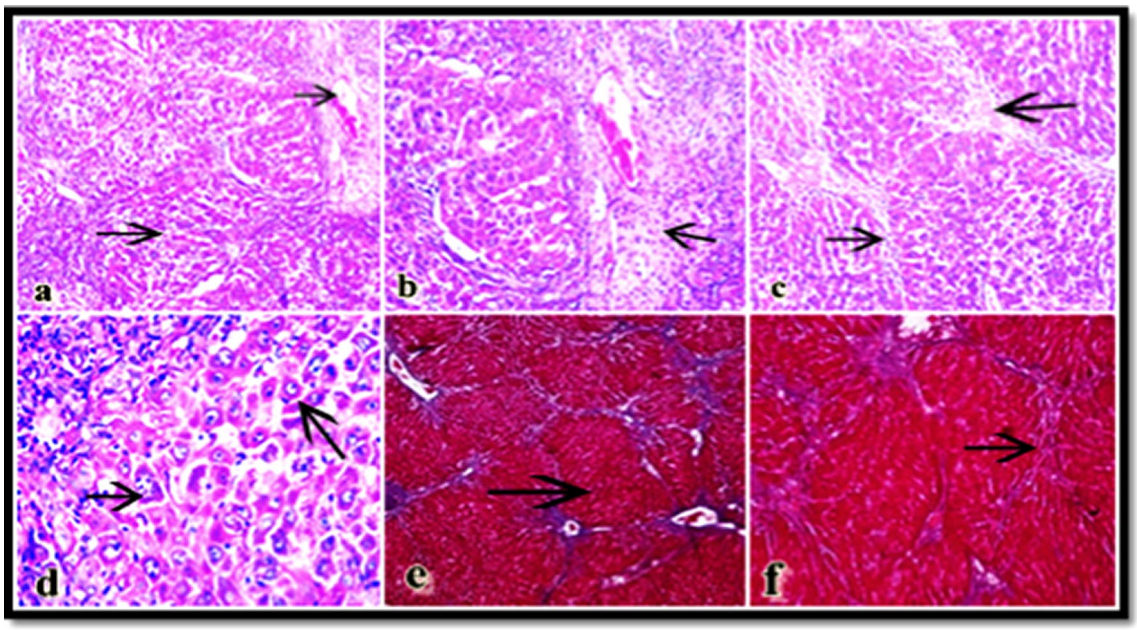


Fig. 8 Liver tissue section from TAA + SOF (group VI) showing: a multinodular liver cirrhosis surrounded by dense fibrous connective tissue **arrow** (×100), **b** dense fibrous tissue proliferation in portal tirade with focal aggregation of lymphocytes and macrophages **arrow** (×200), **c** dense fibrous tissue proliferation in bridging form **arrow** (×100), **d** nuclear pleomorphism with deeply with basophilic scanty cytoplasm and **arrow** (H&Ex400), **e** multiple nodular lesions which consisted of regenerated nodules surrounded with fibrous tissue **arrow** (MTCx100), **f** fibrous tissue bridging between portal triads **arrow** (MTCx200)

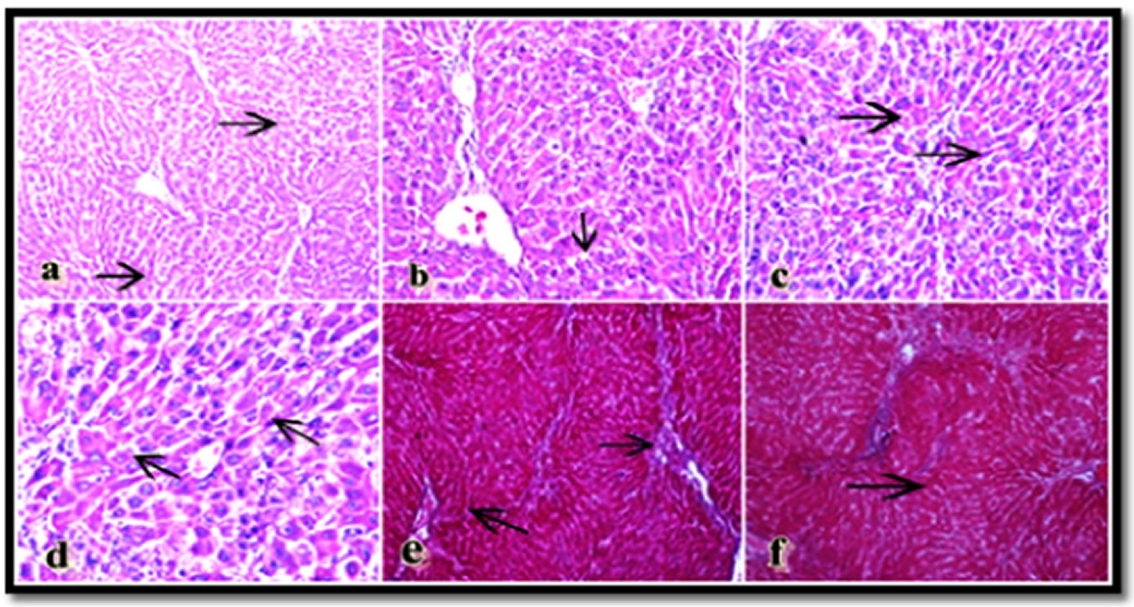


Fig. 9 Liver tissue section from fibrosis model receiving nano-SOF prophylactic (group VII) showing: a disorganization of hepatic cords with degeneration of hepatocytes **arrow** (×100), **b** intracellular fat droplets and delict fibrous connective tissue in portal tirade **arrow** (×200), **c** degeneration of hepatocytes and hyperplasia of Kupffer cells **arrow** (×100), **d** apoptosis of hepatocytes appeared as deeply eosinophilic bodies **arrow** (H&Ex400), **e** delicate fibrous connective tissue proliferation around portal triads (MTCx100), **f** normal architecture of hepatic lobule **arrow** (MTCx100)

To the best of our knowledge, the study is the first to demonstrate the hepatoprotective effects of nano-sofosbuvir in TAA-induced liver injury. This hepatoprotective effect was tested by indirect and direct indicators of liver fibrosis as well as invasive histopathological study of liver tissue.

The present work demonstrated that twice weekly IP injection of TAA for 8 weeks generated severe liver fibrosis—stage 3—F3 and functional lesions in the form of a statistically significant increase in the biochemical markers AST, ALT and ALP with positive reactivity to the fibrosis marker TGF-$\beta_{1.}$

This was in line with the findings of Afifi et al. [27]; Mansour et al. [28] who found that TAA-induced liver fibrosis was accompanied with both functional and histopathological together with the elevation of markers of liver fibrosis.

Thioacetamide is a powerful hepatotoxic drug, used experimentally to provoke acute and chronic liver injury as it affects the protein synthesis, RNA, DNA and gamma-glutamyl transpeptidase activity. It is metabolized in the liver by Cytochrome P450 enzyme which results in the formation of oxidative chains of toxic substances [16]. Increased oxidative stress causes cell death, which in turn triggers an inflammatory response and the activation of HSCs, and finally hepatic cirrhosis [29, 30]. This model mimics key features of human liver fibrosis, including hemodynamic disturbances, morphological changes and biochemical alterations, making it a relevant and reliable system for studying liver disease progression and evaluating potential treatments. Unlike some other fibrosis models, such as those induced by carbon tetrachloride (CCl_4), the fibrosis induced by TAA is more dynamic and resembles the rapid progression of liver damage observed in human liver fibrosis. This makes it an ideal model for investigating hepatoprotective treatments and the potential therapeutic effects of nano-sofosbuvir in reversing or halting the progression of liver fibrosis [31].

The current study revealed that sofosbuvir administration to normal and fibrotic rats resulted in a considerable rise in liver function measures, together with significant increase in tissue TGF-β_1 level. These findings were consistent with Elarabany et al. [32] who reported that sofosbuvir significantly increased serum AST, ALT and ALP levels when given orally to normal rats for 12 weeks both alone and in combination with ribavirin. Mehmood et al. [33] find no variation in serum AST, ALT or ALP levels in normal rats following SOF therapy.

Hosny et al. [34] conducted a study on normal rats to assess the effect of sofosbuvir medication on the histological structure of adult male albino rats' lungs. The mean serum TGF-β1 value significantly increased in the sofosbuvir-treated group compared to the control group, and the sofosbuvir-treated rats' lungs showed changes in both histology and immunohistochemistry.

Moreover, Dyson et al. [35] demonstrated serious drug-induced hepatotoxicity in patients with decompensated hepatitis C fibrosis treated with sofosbuvir and ribavirin.

According to Radaev et al. [36], transforming growth factor beta (TGF-β) isoforms, such as TGF-β1, TGF-β2 and TGF-β3, are homodimeric signaling proteins that are released at a 25 kDa size. TGF-β isoforms function biologically by starting the TGF-β signaling pathway. While TGF-β is crucial for the development of liver disease, including fibrosis, cirrhosis, initial liver injury and hepatocellular carcinoma [37], research has shown that TGF-β inhibits HCV RNA replication and protein expression [38, 39]. Additionally, the three TGF-β isoforms have opposing effects on the development of the fibrosis condition, with TGF-β1 promoting fibrosis and TGF-β3 having an antifibrotic impact [40, 41].

In addition, SOF administration to normal rats resulted in moderate periportal fibrosis—stage 2—F2. These findings agreed with those of Yousefsani et al. [42], who investigated the molecular/cellular mechanisms that contribute to sofosbuvir-induced hepatotoxicity in isolated rat normal hepatocytes. Reactive oxygen species (ROS) generation, mitochondrial membrane potential collapse, lysosomal membrane damage and glutathione depletion were identified as mechanisms of sofosbuvir-induced hepatotoxicity in the research. Moreover, Fanny et al. [43] looked at the cytotoxicity of SOF treatment in HepG2 cell lines and normal hepatocytes. The study discovered that SOF therapy significantly increases proinflammatory cytokines including IL-6 and IL-8, along with a decrease in the cell survival rate of both cancer and normal cells. These results support the hypothesis that SOF treatment induces inflammatory and necrotic processes in treated cells by increasing IL-6 and IL-8 production. These findings, however, were conflicting with Elbakry [44], who found no histological changes following therapy with sofosbuvir and no negative effects in normal rats.

The mechanism by which sofosbuvir might cause liver injury during treatment of HCV patient with fibrosis is not known. Sudden decompensation throughout sofosbuvir therapy may consider changes in the immune status due to the suppression of HCV replication and liver injury [45].

It was reported that two cases had a significant hepatotoxicity related to treatment with NS5A inhibitors and sofosbuvir as a part of the English early access program [35]. In this program, patients with decompensated cirrhosis due to hepatitis C received

12 weeks of treatment with ribavirin, sofosbuvir, and ledipasvir or daclatasvir. Although the link to DAAs has not been proved, these examples suggest that patients with advanced liver disease should be closely monitored while they are on DAA medication, and if there is a major unexplained decline in liver function, DAAs should be stopped.

The administration of galactose-anchored bilosomal formulas (nanoparticles) to normal rats resulted in a significant increase in mean tissue TGF-β_1.

The nanoparticles utilized in the study were created using a thin-film hydration process with stabilizers such as span 60 and sodium taurocholate. A central composite design was used to statistically optimize the provided formulas. The optimized plain and galactosylated formulations have vesicular size, zeta potential and entrapment efficiency of 140–150 nm, − 50 mV and 85%, respectively, and are composed of SAAs (S60 and STC)-to-drug ratio of 1:1 w/w and sodium taurocholate-to-span ratio of 10:1 w/w. The optimized formulations were lyophilized to improve physical stability and allow for more precise medication administration [15]

Ding et al. [46] conducted an 8-week investigation on Nile tilapia fed a basic diet containing sodium taurocholate at 600 mg/kg. They exhibited cholesterol buildup as well as liver fibrosis. Nuclear factor E2-related factor 2 (nrf2) signaling-associated oxidative stress factors were considerably high. Furthermore, greatest level of expression in the liver of genes encoding endoplasmic reticulum (ER) stress and inflammatory cytokines was detected.

Also, too much D-galactose administration accounts for the increased formation of reactive oxygen species, which leads to oxidative stress and hepatocyte damage. Increased lipid peroxidation marker, malondialdehyde (MDA), has been highlighted following D-galactose administration [47].

Meanwhile, SOF encapsulated in galactose-anchored bilosomal formulas, given prophylactic to TAA-treated rats aiming to investigate the abilities of sofosbuvir to target the liver, resulted in significant amelioration of TAA-induced liver injury that was evident by the reduction in serum level of AST, ALT and ALP, and also improvement of liver histopathological changes compared to TAA group and TAA–SOF-treated group.

Using sofosbuvir therapeutically presents several difficulties, chief among them being its low bioavailability and restricted liver targeting. When administered orally, SOF experiences low absorption in the gastrointestinal tract as it is a P-glycoprotein (P-gp) substrate, which causes its efflux from GIT membrane cells and hepatocytes while also inhibiting its cellular internalization [48]. It is widely dispersed throughout the body, resulting in a variety of adverse effects and maintaining its level in the target organ within a small range [7, 49]. Therefore, it is thought that sofosbuvir would be an excellent applicant for liver targeting, which would improve its efficacy, availability and duration of residence in the liver.

The nanoparticle formulation may provide more controlled and localized delivery of sofosbuvir, minimizing the dose-related liver damage typically seen with the free drug. Also, it can improve the drug's bioavailability and targeting to the liver while potentially reducing systemic toxicity. The use of galactose-anchored bilosomes as nanocarriers could facilitate the selective uptake of nano-SOF by liver cells [50]. In addition, the span carrying nanocarriers have the capacity to block efflux pumps (P-gp), which act as a suppressor of hepatocyte drug accumulation [51]. These lead to enhanced therapeutic effects without significant damage to the liver and reduced the release of liver enzymes (AST, ALT and ALP) [50]. The decreased levels of these biomarkers suggest that nano-SOF's targeted delivery may reduce the hepatotoxicity often associated with traditional sofosbuvir administration, resulting in less liver injury and a more favorable therapeutic outcome.

Moreover, when SOF encapsulated in galactose-anchored bilosomal formulas, given prophylactic to TAA-treated rats, it resulted in significant reduction in tissue TGF-β_1.

The galactose moiety in galactosylated taurocholate bilosomal facilitates the specific uptake of these bilosomes by hepatocytesvia the ASGPR. By attaching therapeutic agents (such as antifibrotic drugs, antioxidants or gene therapies) to these bilosomes, it becomes possible to deliver drugs directly to the liver, minimizing systemic side effects and improving efficacy [52]. The taurocholate component of the bilosome enhances penetration and retention of the drug inside the liver [53]. Bilosomes can be loaded with antifibrotic agents that target the underlying molecular pathways causing fibrosis and potentially halt or reverse fibrosis progression. It can also carry drugs that reduce liver inflammation and oxidative stress, and help alleviate the fibrotic process by addressing the underlying inflammatory and oxidative damage. Additionally, DNA- or RNA-based therapy targeting genes involved in fibrosis can be encapsulated in bilosomes for targeted delivery to the liver. This can include siRNAs (small interfering RNAs) that silence profibrotic genes or genes that promote liver repair [54].

The hepatoprotective role of SOF encapsulated in galactose-anchored bilosomes is based on targeted delivery to hepatocytes, enhanced bioavailability and the reduction of liver inflammation and fibrosis. The formulation improves the therapeutic potential of SOF

by preventing hepatocyte damage, reducing oxidative stress and modulating proinflammatory pathways. The dual targeting mechanism with galactose and bile salts further enhances liver-specific drug delivery, making this formulation a promising candidate for the treatment of liver diseases, particularly those related to HCV infection and liver fibrosis. Future studies are needed to explore the full molecular mechanisms and long-term effects of this formulation in both preclinical and clinical settings [55, 56].

The study's potential limitations

Although the TAA model shares similarities with human liver fibrosis, there are differences in the rate of disease progression and response to treatment [57]. Additionally, human liver fibrosis involves complex interactions between genetic, environmental and metabolic factors, which may not be fully replicated in a rat model [58]. Therefore, while the rat model is valuable for preclinical studies, the results should be interpreted cautiously when considering human applications.

The observed hepatoprotective effects of nano-sofosbuvir could be influenced by several confounding factors. For instance, the dosage of SOF, the route of administration and the duration of treatment can all impact the outcomes. Furthermore, the bioavailability and targeted delivery of nano-sofosbuvir in the liver may be influenced by various physiological conditions, such as hepatic blood flow, liver enzyme activity and drug interactions with other substances in the liver. These factors should be considered when interpreting the effectiveness of nano-sofosbuvir in protecting against liver fibrosis. Although nano-sofosbuvir represents a promising approach to improving the delivery of SOF, the nanoformulation itself may have certain limitations, such as variability in particle size, stability of the formulation and the potential for immune system recognition or clearance by the liver, which may affect the overall efficacy [59]. Further optimization of the bilosomal formulation is needed to ensure consistent delivery and reduced immunogenicity. Given the complexity of liver diseases and the various factors influencing treatment efficacy, future studies should aim to address these limitations through longer-term studies, different animal models and clinical trials. This would help establish the full potential and applicability of nano-sofosbuvir in the context of human liver diseases.

Conclusion

The research findings strongly support the concept that galactosylated taurocholate bilosomal represent a promising nanocarrier for the targeted delivery of sofosbuvir (SOF) to the liver. However, further studies are needed to explore the molecular mechanisms underlying the hepatoprotective effects of SOF encapsulated in galactose-anchored bilosomes. Future research should focus on optimizing nanoparticle formulations, targeting techniques and therapy schedules to enhance treatment efficacy and safety. Key areas for investigation include pharmacokinetics, long-term effects and the most effective drug delivery strategies for clinical use.

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ASGPR	Asialoglycoproteins
AST	Aspartate aminotransferase
CCL_4	Carbon tetrachloride
DAAs	Direct acting antivirals
DMSO-d6	Dimethyl sulfoxide_d6
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
FTIR	Fourier transform infrared
HCV	Hepatitis C virus
HCV RNA	Hepatitis C virus ribonucleic acid
H&E	Hematoxylin and eosin
HepG2 cell lines	Human hepatocellular carcinoma cell line
HSCs	Hepatic stellate cells
IL-6	Interleukin-6
IL-8	Interleukin-8
IP	Intraperitoneal
LOBF	Lyophilized optimized liposomal formula
MDA	Malondialdehyde
Nano-SOF	SOF encapsulated in galactosylated taurocholate bilosomal formula
NPs	Nanoparticles
nrf2	Nuclear factor E2-related factor 2
NS5B	A non-structural protein 5B
PBS	Cold phosphate saline
PC	L-a-Phosphatidylcholine
P-gp	P-glycoprotein
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SAAs	Span 60 and STC
SD	Standard deviation
SOF	Sofosbuvir
SPSS	Statistical Package for the Social Sciences
STC	Sodium taurocholate
S60	Span 60
TAA	Thioacetamide
TGF-β	Transforming growth factor beta

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Author contributions

All authors contributed to the study conception and design. Material preparation were performed by M J and AM. Methodology, data collection and analysis were performed by M KM, MNM, ANS and A H. O. The first draft of the manuscript was written by MNM and M KM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The current study was approved by the Institutional Animal Care and Use Committee, Cairo University (IACUC) (approval No: CU/III F9119).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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