Optimal dose of silymarin for the management of drug-induced liver injury in oncology

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Abstract. Systemic oncological treatment may cause drug-induced liver injury (DILI). Therefore, there is a pressing need for an active drug able to accelerate liver regeneration. Silymarin mitigates oxidative stress, and inhibits pro-inflammatory and pro-apoptotic cytokines and the fibrotic transformation of liver tissue. Currently, there are a lack of data regarding the optimal dosage of silymarin and its efficacy. Thus, the present retrospective study aimed to determine the optimal dose of silymarin for use in oncological DILI treatment. For this purpose, 180 patients with solid malignancies treated with systemic oncological therapy and silymarin between January, 2015 and November, 2021 were enrolled in the study. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (Bil) levels, as well as the dose of silymarin were assessed at the initiation of silymarin treatment, after 3-6 weeks and after 6-12 weeks. Pearson's correlation analysis was performed to evaluate the correlation between the initial dose of silymarin (IDoS), and the ALT, AST and Bil levels. The effects of four independent variables, namely IDoS, the initial dose reduction of systemic treatment, the systemic treatment dose reduction at first assessment (DR1M) and the elevation of the silymarin dose at first control on the ALT, AST and Bil levels were evaluated using regression analysis. The median IDoS was 450 mg. A decrease in or the stabilization of the ALT, AST and Bil levels after 6-12 weeks were observed in 68.63, 65.85 and 53.25% of patients, respectively. There was a weak correlation between IDoS and the decrease in ALT and AST levels after 6-12 weeks (correlation coefficient, R=0.361 and 0.277 respectively, P<0.001). No significant correlation between the IDoS and a decrease in Bil levels was observed. DR1M was a negative predictor for a decrease in Bil levels in patients with liver tumors. On the whole, the present study demonstrates that silymarin appears to be efficient in alleviating DILI at a dose of 300-450 mg. A further increase in the dose of silymarin may not lead to an adequate increase in its efficacy.

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Introduction

The liver is a parenchymatous organ that is essential for intermediate metabolism and detoxication. Hepatocytes are the main cells in the liver, representing almost 80% of the whole liver mass (1). Despite the strong regenerative potential of the liver, the continuous or repeated intake of several drugs can result in liver injury. This phenomenon is commonly known as drug-induced liver injury (DILI). As with any other drugs used for other purposes, the liver is the first site of biotransformation during the systemic treatment of cancer. The mechanisms of liver damage vary with particular cytostatics and their combinations, and detailed data on the epidemiology of chemotherapy-induced liver injury (CILI) are insufficient (2).

There are several strategies used for the management of CILI. Dose reduction or a delay in administering chemotherapy are common strategies used to combat an impaired hepatic function. However, both strategies may lead to a decrease in the efficacy of systemic treatment in terms of lowering the survival rates (3,4). Therefore, there is an urgent need for the development of an active drug that can accelerate liver regeneration.

Silymarin is an extract of milk thistle (*Silybum marianum)*, which consists of a mixture of flavonolignans, flavonoids and polyphenols. Silybinin is the dominant and most biologically active compound present in silymarin. The effect of silymarin on the liver is pleiotropic. The excessive intake of toxins or intensive oxidation as a part of free fatty acid metabolism results in the elevated production of reactive oxygen species. Silymarin mitigates this oxidative stress by scavenging reactive oxygen species (5). The anti-inflammatory effects mediated by the inhibition of a number of pro-inflammatory cytokines (such as IL-1, IL-6 and TNF-α) have been previously documented by Federico *et al* (6). The downregulation of oxidative and inflammatory activity results in a decreased level of parenchymal damage. Silymarin also inhibits the conversion of stellate cells into myofibroblasts and reduces the production of procollagen III, α-SMA and TGF-β. Along with the anti-apoptotic effect of silymarin, the inhibition of the fibrotic transformation of liver tissue is also observed (6).

In general, there are limited data available on the efficacy of silymarin in DILI. The majority of trials focus on liver toxicity induced by antituberculotic drugs. Luangchosiri *et al* (7) reported that silymarin at a dose of 420 mg daily decreased the incidence of DILI associated with antituberculotic treatment. However, in another randomized controlled trial by Marjani *et al* (8), silymarin failed to exhibit similar activity in alleviating already developed DILI following the intake of antituberculotic drugs (8).

Even less data are available regarding the efficacy of silymarin in the management of CILI. The randomized prospective trial by Moezian *et al* (9) investigated the role of silymarin in 30 patients with early-stage breast cancer treated with the chemotherapy regimen, AC/T (doxorubicin, cyclophosphamide followed by paclitaxel) who developed radiologically-confirmed CILI during/after chemotherapy. Silymarin at a dose of 140 mg daily failed to lead to a statistically significant improvement in the levels of biochemical and radiological markers of liver injury in the experimental arm. Nevertheless, they concluded that there was a trend in favor of the use of silymarin. Acknowledging the limitations of this trial, Moezian *et al* (9) called for further investigations in this matter.

Another trial by Mohaghegh *et al* (10) focused on the effects of silymarin on taxane-based CILI. In their study, 99 patients with invasive breast cancer treated with anthracyclines followed by taxanes (docetaxel or paclitaxel) were randomly divided into two study arms. The patients in the experimental arm were administered silymarin at a dose of 70 mg three times a day during chemotherapy. The control arm was administered a placebo. The levels of two liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] and serum bilirubin (Bil) levels were measured after each dose of taxane (10). Although there was initially only a small difference in the levels of liver enzymes and Bil between the groups, a statistically significant difference between the groups was observed in the levels of liver enzymes after 1 month of treatment. They thus concluded that silymarin can revoke the increase in the levels of liver enzymes when administered to patients treated with taxanes, and stated that this effect may be enhanced with higher doses of silymarin (10).

Inconsistent results may originate from the various doses of silymarin used in the cited trials. The optimal dosage of silymarin is a matter of debate. Fathalah *et al* (11) reported improved outcomes in patients treatment for liver injury with high-dose silymarin (1,050 mg daily) compared to a 'standard' dose of silymarin (420 mg daily) in patients with decompensated liver cirrhosis. That trial suggests that the effectivity of silymarin on liver injury may be dose-dependent (11).

The present study retrospectively examined 180 patients treated with systemic oncological treatment (chemotherapy and/or targeted therapy) who were also treated with silymarin due to an elevation in the levels of liver enzymes and/or Bil. The present study aimed to assess the association between the dose of silymarin and a decrease in the levels of selected liver function parameters. The goal was to determine whether an increased dose of silymarin is associated with improved liver function parameters and subsequently, to assess the optimal dose of silymarin for the treatment and prevention of CILI.

Patients and methods

Patient information. Adult patients with solid malignancies, including lymphomas treated with systemic oncological therapy and silymarin between January, 2015 and November, 2021 were included in the present retrospective study. Both male and female patients were included. Patients with normal and elevated levels of liver markers, such as ALT, AST and Bil in liver function tests (LFTs) were enrolled in the present study. No patients with known viral hepatitis were included in the study. There were no other selection criteria regarding diagnosis, stage, age, systemic treatment or the dose of silymarin. The characteristics of the included patients are presented in Table I. The present study was approved by the Ethics Committee of Faculty Hospital Trencin (Trencin, Slovakia). Since the present study was retrospective and non-interventional in nature, the requirement to obtain patient informed consent for participation was waived by the Ethics Committee.

Assessment of transaminases and Bil. The levels of transaminases (ALT and AST) and total serum Bil levels were assessed prior to the initiation of silymarin use, and at 3-6 weeks (1st assessment; ALT1, AST1 and Bil1) and at 6-12 weeks (2nd assessment; ALT2, AST2 and Bil2) following the initiation of treatment. Concurrently, the dose of silymarin was determined at the initiation of treatment, and at the 1st and 2nd assessment. In the case of incomplete data for AST, ALT or Bil levels (missing 1st or 2nd assessment), the patient was censored and LFTs with incomplete data were not evaluated. The reference values for ALT, AST and Bil were 0.05-0.75, 0.05-0.63 μkat/l and 3.0-17.0 μmol/l, respectively.

An Architect® ci16200 analyzer (Abbott Pharmaceutical Co. Ltd.) was used for the determination of the serum ALT, AST and Bil levels. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)-approved method with nicotinamide adenine dinucleotide hydrogen and pyridoxal 5'-phosphate was used for the evaluation of the ALT and AST levels. The serum Bil level was determined using the IFCC-approved diazonium salt method. The reagents were supplied by Abbott Pharmaceutical Co. Ltd.

A >20% elevation from the baseline values in the LFTs was considered as an increase. A decrease >20% of baseline value was considered as a decrease. Any change in LFT values that was <20% of the baseline value was regarded as stabilization. Any change that was within the normal (reference) range of values for a particular LFT was considered a stabilization.

Determination of the association between the dose of silymarin and the levels of transaminases and Bil. Pearson's correlation analysis was performed to evaluate the association between the initial dose of silymarin, and the levels of transaminases and Bil. Subsequently, three independent variables were established based on collected data and common medical practice when dealing with liver toxicity. These variables were named 'initial dose reduction of systemic treatment' (IDR), 'systemic treatment dose reduction at first assessment' (DR1M) and 'increase of the silymarin dose at first assessment' (SDE).

The IDR was defined as the absolute difference between the standard dose of systemic treatment (either calculated

Table I. Clinicopathological characteristics of the 180 patients included in the present study.

Clinicopathological characteristics	Values
Age, years	
Average	63.3
Median	64.55
Range	22.07-93.05
Sex, n (%)	
Male	97 (53.89)
Female	83 (46.11)
Cancer subtypes, n (%)	
Adenocarcinoma	162 (90)
Squamous cell cancer	6 (3.33)
Non-Hodgkin lymphoma	4 (2.22)
Neuroendocrine tumor	3 (1.67)
GIST	2 (1.11)
Small cell cancer	1 (0.56)
High-grade glioma	1 (0.56)
Non-seminoma	1 (0.56)
Stage at the time of diagnosis, n (%)	
I	11 (6.11)
II	22 (12.22)
III	60 (33.33)
III	87 (48.33)
Stage at the time the initiation of silymarin treatment, n (%)	
I	5 (2.79)
II	12 (6.67)
III	37 (20.55)
IV	126 (70)
Grade, n (%)	
1	43 (23.89)
2	68 (37.78)
3	65 (36.11)
4	2 (1.11)
Non-applicable, n (%)	2 (1.11)
Liver tumor	
Any	77 (42.78)
Liver metastases	73 (40.56)
Primary liver tumor	4 (2.22)
Initial elevation of liver function test, n (%)	
ALT	111 (61.67)
AST	116 (64.44)
Bilirubin	86 (47.78)

Adenocarcinoma includes colorectal adenocarcinoma, invasive ductal adenocarcinoma of the breast, invasive lobular adenocarcinoma of the breast, pancreatic ductal adenocarcinoma, renal cell adenocarcinoma, adenocarcinoma of the lung, stomach adenocarcinoma, cholangiocarcinoma and ovarian serous adenocarcinoma. GIST, gastrointestinal stromal tumor.

according to body surface area/weight or flat-fixed dose) and the actual dose of systemic treatment at the start of silymarin use. DR1M was defined as the absolute difference between the standard dose of systemic treatment (either calculated according to body surface area/weight or flat-fixed dose) and the actual dose of systemic treatment at 1st assessment (mentioned above). The SDE was defined as the absolute difference between the initial dose of silymarin and the actual dose of silymarin at 1st assessment.

Finally, the effects of the initial dose of silymarin (IDoS), IDR, DR1M and SDE (independent variables) on the levels of ALT2, AST2 and Bil2 (dependent variables) were evaluated. These effects were evaluated in a population of patients with livers tumor and without liver tumors.

Statistical analysis. The initial evaluation of the correlation between the IDoS, and the values of ALT, AST and Bil was performed using Pearson's correlation analysis. Subsequently, in order to detect potential confounding factors in the association between the IDoS and the results of the LFTs, regression analysis was performed. Regression analysis was performed in a subpopulation of patients with liver lesions and without liver lesions. Since the independent variables were non-parametric (continuous data), multiple linear regression was used to evaluate the effects of IDoS, IDR, DR1M and SDE on the levels of ALT2, AST2 and Bil2. Python version 3.9.5 (Python Software Foundation, 2021) and IBM SPSS Statistic v 28.0 (IBM, 2021) were used for data processing.

Results

A total of 654 patients were screened in the initial research. Subsequently, 180 patients (83 females and 97 males) were included in the retrospective study. The median age of the patients was 64.55 years. The most frequent diagnosis was that of colorectal cancer (n=77, 42.78%) followed by breast (n=24, 13.33%) and pancreatic cancer (n=16, 8.89%). The baseline characteristics of the patients according to diagnosis are presented in Fig. 1.

At the time of diagnosis, 87 (48.33%) patients had metastatic cancer, 60 (33.33%) patients had stage III cancer, stage II was present in 22 (12.22%) patients and 11 (6.11%) patients had stage I cancer. At the time of the initiation of silymarin treatment, 126 (70%) patients had stage IV cancer, 37 (20.55%) had stage III cancer, 12 (6.67%) patients presented with stage II cancer and 5 (2.79%) patients had stage I cancer. Liver metastases were present in 73 (40.56%) patients and 4 (2.22%) patients were treated for primary liver tumors. The clinicopathological characteristics of the included patients are presented in Table I.

An initial elevation in ALT levels was documented in 111 (61.67%) patients and an elevation in AST levels was found in 116 (64.44%) patients. On the other hand, less than half of the patients presented with an elevation in total serum Bil levels (86, 47.78%) (Table I). In total, 111 (61.67%) patients were treated with chemotherapy, 38 (21.11%) patients were treated with combined chemotherapy and targeted therapy, and 31 (17.22%) patients were treated with targeted therapy only. The characteristics of the patients according to the systemic therapy used are presented in Fig. 2. The median initial dose of chemotherapy was 75%; thus, the median dose reduction was 25%. Since no clear recommendation regarding the dosage

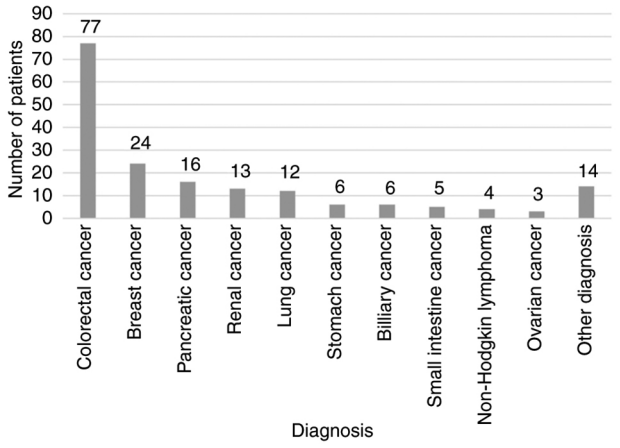

Figure 1. Characteristics of the patients according to diagnosis. Diagnoses marked as 'Other' contributed with <3 cases to the present study.

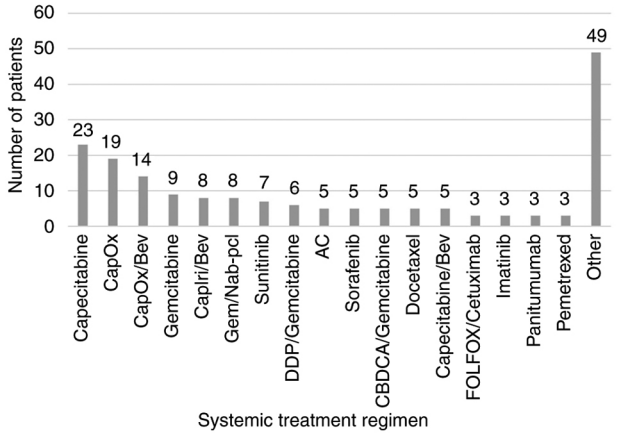

Figure 2. Characteristics of patients according to the chemotherapy used. CapOx, capecitabine/oxaliplatin; Bev, bevacizumab; CapIri, capecitabine/irinotecan; Gem/Nab-pcl, gemcitabine/nab-paclitaxel; DDP, cisplatin; AC, adriamycin/cyclophosphamide; CBDCA, carboplatin; FOLFOX, folinic acid, 5-fluoruracil, oxaliplatin. Regimens marked as 'Other' contributed with <3 cases to the present study.

of silymarin is available, the initial dose of silymarin was at the discretion of the physician and ranged from 150-900 mg. However, the majority of the patients were treated with a dose of 450 mg (n=101, 56%) and 300 mg (n=63, 35%). The median initial dose of silymarin was 450 mg. The sum of patients who achieved stabilization or a decrease in the levels of transaminases and Bil is presented in Table II.

A weak to moderate correlation was observed between the IDoS and a decrease in the levels of transaminases. The results of Pearson's correlation analysis are presented in Table III. This correlation was, however, statistically significant in both cases (P<0.001). No statistically significant correlation was observed between the Bilirubin levels and the IDoS. Regression analysis confirmed that the IDoS was a statistically significant positive predictor for a decrease in ALT2 levels either in patients with liver lesions or without liver lesions. This effect appears to be stronger in patients with liver lesions.

Similarly, IDoS was a positive predictor for a decrease in AST2 levels in patients with and without liver lesions. Again, the effect was more pronounced in the population of patients with liver lesions. Notably, the DR1M appeared to be a negative predictor for a decrease in AST2 levels in patients without liver lesions with borderline statistical significance. Finally, IDoS was not observed to be a positive predictive factor for a decrease in Bil levels. However, similar to AST2, the DR1M was a negative predictive factor of a decrease in Bil levels in patients with liver lesions. The complete results of the regression analyses for the association between IDoS, DR1M and SDE, and the levels of transaminases and Bil are presented in Tables IV-VI.

Discussion

The present retrospective study demonstrated that silymarin was effective in reducing or stabilizing the ALT, AST and Bil levels in more than half of the patients included (67.46, 62.8 and 50.89%, respectively). Although an increase was observed in the levels of transaminases (31.37% for ALT and 34.15% for AST) and Bil (46.75%) in a large portion of patients treated with silymarin, it could not be concluded that silymarin was not effective in these patients. Silymarin could have mitigated the detrimental effects on LFTs caused by other factors (e.g., liver lesion progression, other medication, unidentified underlying liver conditions, etc.). These factors need to be identified and evaluated in future prospective trials in selected populations.

The present study observed some association between the IDoS and a decrease in the levels of transaminases. Correlation coefficients expressing the correlation between increasing the initial dose of silymarin and a decrease in ALT and AST levels were low (R=0.333 and 0.276, respectively at the 1st assessment; and R=0.361 and 0.277, respectively at the 2nd assessment). Although the P-values of correlation models for both transaminases suggest statistical significance, the impact of the IDoS on the levels of transaminases appears to be low. These results were confirmed in the regression analysis. Despite the fact that the present study was retrospective in nature and only a limited number of medical records were available, three independent variables were established based on the available collected data and common medical practice when dealing with liver toxicity (initial dose reduction of systemic treatment, systemic treatment dose reduction at first control, the elevation of silymarin dose at first assessment). It was hypothesized that these three independent variables may have affected the association between the IDoS and the decrease in the values of parameters in the LFTs. This presumption appears to have been mostly wrong.

Neither the IDR nor the SDE had any significant effect on the ALT2 and AST2 levels. However, it should be taken into consideration that the elevation in the silymarin dose was done due to an unsatisfactory decrease in the levels of transaminases and/or Bil after first period of treatment. While there is only a weak association between increasing the IDoS and its effect on the levels of transaminases, there may be other factors diminishing the effect of silymarin e.g., a patient's unresponsiveness to silymarin or a higher level of systemic therapy toxicity.

The impact of DR1M on AST2 is questionable. A borderline statistically significant association between DR1M and AST2 was observed. This result suggests that the dose reduction of systemic treatment would diminish the effect of silymarin on the reduction of the AST level. However, this was

Table II. Characteristics of the patients who achieved a decrease, stabilization or an increase in parameters in LFTs during treatment with silymarin.

LFT	Decrease, n (%)	Stabilization, n (%)	Increase, n (%)	Censored, no. of patients
ALT	114 (67.46)	2 (1.18)	53 (31.37)	11
AST	103 (62.8)	5 (3.05)	56 (34.15)	16
Bil	86 (50.89)	4 (2.37)	79 (46.75)	11

Patients with incomplete data for AST, ALT or Bil (missing 1st or 2nd assessment) were censored. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bil, bilirubin; LFT liver function test.

Table III. Outcomes of Pearson's correlation analysis between the initial dose of silymarin and the parameters from LFTs.

Predictive factor	LFT	Pearson's correlation coefficient (R value; LFT)	Significance (two-tailed)	No. of patients
Initial dose of silymarin	ALT (1st assessment)	0.333	<0.001	179
Initial dose of silymarin	ALT (2nd assessment)	0.361	<0.001	169
Initial dose of silymarin	AST (1st assessment)	0,276	<0.001	175
Initial dose of silymarin	AST (2nd assessment)	0.277	<0.001	164
Initial dose of silymarin	Bil (1st assessment)	-0.015	0.842	180
Initial dose of silymarin	Bil (2nd assessment)	-0.009	0.910	169

Pearson's correlation analysis was performed to assess the correlation between the initial dose of silymarin and particular LFT parameters. Increasing the initial dose of silymarin proved to have a weak association with a decrease in the ALT and AST levels. No statistically significant correlation was found between increasing the initial dose of silymarin and a decrease in Bil levels. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bil, bilirubin; LFT liver function test.

Table IV. Outcomes of the regression analysis on the effects of IDoS, SDE, IDR and DR1M on a decrease in the ALT level at the 2nd assessment.

Dependent variable	Liver lesion	Independent variable	Std Beta	t-value	P-value	R^2 value
ALT (2nd assessment)	N	IDoS	0.221	2.111	0.037	0.057
ALT (2nd assessment)	N	SDE	-0.030	-0.290	0.772	0.057
ALT (2nd assessment)	N	IDR	-0.006	-0.052	0.959	0.057
ALT (2nd assessment)	N	DR1M	-0.089	-0.818	0.416	0.057
ALT (2nd assessment)	Y	IDoS	0.453	4.196	0.001	0.215
ALT (2nd assessment)	Y	SDE	-0.105	-0,972	0.334	0.215
ALT (2nd assessment)	Y	IDR	-0.107	-0.883	0.380	0.215
ALT (2nd assessment)	Y	DR1M	0.073	0.597	0.552	0.215

In Pearson's correlation analysis, the IDoS was proven to have a weak, yet statistically significant correlation with a decrease in ALT and AST levels. The regression analysis was performed in an aim to determine the effects of IDoS, SDE, IDR and DR1M on ALT levels at the time of the 2nd assessment both in patients with liver tumors and without liver tumors. Only IDoS was proven to have a statistically significant effect on ALT at the time of the 2nd assessment. Values in bold font indicate a statistically significant difference (P<0.05). ALT, alanine aminotransferase; IDoS, initial dose of silymarin; SDE, elevation of silymarin dose at first assessment; IDR, initial dose reduction of systemic treatment; DR1M, systemic treatment dose reduction at first assessment; N, no; Y, yes.

observed in a subpopulation of patients with no primary or secondary liver tumors. There was no significant association between DR1M and AST2 in patients with liver tumors. The mechanism behind this finding remains unknown.

No any association was observed between increasing the IDoS and a decrease in Bil levels. However, in the regression analysis, the DR1M had a significantly negative impact on the decrease in the Bil level. This negative impact was observed only in patients with primary liver tumors or liver metastases. It was hypothesized that this effect may have been caused by the decreased efficiency of systemic treatment delivered at a reduced dose. This may have resulted in

Table V. Outcomes of the regression analysis of the effects of IDoS, SDE, IDR and DR1M on a decrease in the AST level at the 2nd assessment.

Dependent variable	Liver lesion	Independent variable	Std Beta	t-value	P-value	R^2-value
AST (2nd assessment)	N	IDoS	0.279	2,684	0.009	0.110
AST (2nd assessment)	N	SDE	-0.008	-0.080	0.936	0.110
AST (2nd assessment)	N	IDR	-0.107	-0.980	0.330	0.110
AST (2nd assessment)	N	DR1M	-0.221	-2.015	0.047	0.110
AST (2nd assessment)	Y	IDoS	0.312	2.690	0.009	0.107
AST (2nd assessment)	Y	SDE	-0.110	-0.948	0.347	0.107
AST (2nd assessment)	Y	IDR	-0.046	-0.355	0.724	0.107
AST (2nd assessment)	Y	DR1M	0.065	0.496	0.621	0.107

In Pearson's correlation analysis, the IDoS was proven to have a weak, yet statistically significant correlation with a decrease in ALT and AST levels. The regression analysis was performed in an aim to determine the effects of IDoS, SDE, IDR and DR1M on AST levels at the time of the 2nd assessment both in patients with liver tumors and without liver tumors. Only IDoS was proven to have a statistically significant effect on AST at the time of the 2nd assessment. In patients without liver tumors, DR1M appeared to have a borderline statistically significant negative impact on decrease in AST level (2nd assessment). Values in bold font indicate a statistically significant difference ($P<0.05$). AST, aspartate aminotransferase; IDoS, initial dose of silymarin; SDE, elevation of silymarin dose at first assessment; IDR, initial dose reduction of systemic treatment; DR1M, systemic treatment dose reduction at first assessment; N, no; Y, yes.

Table VI. Outcomes of the regression analysis on the effects of of IDoS, SDE, IDR and DR1M on Bil levels at the 2nd assessment.

Dependent variable	Liver lesion	Independent variable	Std Beta	t-value	P-value	R^2-value
Bil (2nd assessment)	N	IDoS	0.148	1.440	0.153	0.086
Bil (2nd assessment)	N	SDE	-0.203	-1.979	0.051	0.086
Bil (2nd assessment)	N	IDR	0.112	1.049	0.297	0.086
Bil (2nd assessment)	N	DR1M	-0.039	-0.368	0.714	0.086
Bil (2nd assessment)	Y	IDoS	0.016	-0.142	0.887	0.141
Bil (2nd assessment)	Y	SDE	-0.093	-0.816	0.417	0.141
Bil (2nd assessment)	Y	IDR	-0.237	-1.872	0.065	0.141
Bil (2nd assessment)	Y	DR1M	-0.380	-2.980	0.004	0.141

In Pearson's correlation analysis, the IDoS was proven to have a weak, yet statistically significant correlation with a decrease in ALT and AST levels, but not with the Bil levels. The regression analysis was performed in an aim to determine the effects of IDoS, SDE, IDR and DR1M on Bil levels at the time of the 2nd assessment both in patients with liver tumors and without liver tumors. Only DR1M in patients with liver tumors was proven to have statistically significant impact on the Bil level at the time of the 2nd assessment. Values in bold font indicate a statistically significant difference ($P<0.05$). Bil, bilirubin; IDoS, initial dose of silymarin; SDE, elevation of silymarin dose at first assessment; IDR, initial dose reduction of systemic treatment; DR1M, systemic treatment dose reduction at first assessment; N, no; Y, yes.

growth of liver tumors and in the promotion of liver damage in some patients. The levels of serum bilirubin may have been under the influence of other confounding factors not covered by the present regression analysis. Moreover, serum Bil was used as a marker of liver tissue function. However, due to insufficient data, the present study did not evaluate conjugated Bil in the patients. Thus, there could be a substantial portion of patients with cholestasis of various etiologies. Although there is limited evidence available on the positive effect of silymarin on drug-induced cholestasis in mouse models (12), the authors were not able to assess the efficacy of silymarin in the case of cholestasis caused by extrahepatic factors e.g., bile duct obstruction.

The present study has several limitations which should be mentioned. Firstly, patients with a wide variety of underlying liver conditions may have been included in the study. Given the fact that the patients were evaluated retrospectively, only incomplete health records were available for consideration. Moreover, patients with initially elevated results in LFTs were enrolled. The present study focused on the dynamics of LFTs during silymarin treatment regardless of the initial value. Finally, malignant liver lesions (primary or metastatic liver tumors) can considerably affect the outcomes of hepatoprotective treatment. Therefore, the results presented in Tables III-V confront two subpopulations of the patients: Those with liver lesions and those without liver lesions.

Of note, in the present study, the R^2 value of the regression model was low. This was caused by a high variability in the data, despite a considerably large set of patients. The dataset was derived from patients with various diagnoses, at various

stages of disease and, most importantly, treated with a wide variety of systemic treatment.

In conclusion, the findings of the present study suggest that the most prevalent initial dose of silymarin (300-450 mg) appears to be sufficient for the treatment of CILI and a higher initial dose of silymarin brings only a limited benefit. Furthermore, based on the data obtained, it could not be determined whether a lower initial dose of silymarin would result in comparable effectivity. In addition, the further escalation of the silymarin dose at first assessment (after 1 month of treatment) cannot be recommended. To the best of our knowledge, this is the first study aiming to determine the optimal dose of silymarin in CILI using the association between the IDoS and the levels of parameters from LFTs. Considering the aforementioned limitations of the present study, further investigations using randomized controlled trials are warranted.

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Availability of data and materials

The data generated in the present study are not publicly available due to legal restrictions applied in Slovakia but may be requested from the corresponding author.

Authors' contributions

FK and BB contributed to the retrospective study by performing data collection and evaluation. Statistical analysis and the final preparation of the manuscript were performed by FK. FK and BB confirm the authenticity of all the raw data. Both authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Faculty Hospital Trencin (Trencin, Slovakia). Since the present study was retrospective and non-interventional in nature, the requirement to obtain patient informed consent for participation was waived by the Ethics Committee.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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