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Original paper

The effect of silymarin on liver enzymes and antioxidant status in trauma patients in the intensive care unit: a randomized double blinded placebo-controlled clinical trial

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Abstract

Aim of the study: This study was conducted to investigate the positive effect of silymarin on liver enzymes and antioxidant status in trauma patients with elevated liver enzymes due to trauma-induced liver injury, admitted to the intensive care unit.

Material and methods: This one-year, randomized, double-blinded, placebo-controlled clinical trial was conducted on 90 trauma patients. The participants were assigned to either receiving Livergol tablets containing 140 mg of silymarin or 140 mg of placebo three times daily for 14 days. Liver enzymes, including aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), were measured at baseline and days 3, 7, 9 and 14 after intervention. Also, antioxidant markers were measured at baseline and day 14 after treatment.

Results: Receiving silymarin supplement significantly lowered the liver enzymes, compared to placebo ($p < 0.05$). The mean serum level of malondialdehyde (MDA) was significantly decreased and the mean serum levels of total antioxidant capacity (TAC) and thiol groups were significantly increased in the silymarin group from baseline to day 14. In the placebo group, mean serum levels of MDA and thiol groups were significantly increased, while serum level of TAC was not significantly changed at day 14, compared to baseline. Also, the mean serum level of MDA was significantly lower, while the serum levels of thiol groups and TAC were significantly higher in the silymarin group.

Conclusions: Silymarin supplementation significantly improved some antioxidant markers (TAC and thiol) and decreased liver enzymes in patients with trauma-induced liver injury.

Key words: liver enzymes, antioxidant status, trauma, silymarin, liver injury.

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Introduction

Liver injury is the most common organ damage in patients with blunt multiple traumas, as well as mild abdominal trauma [1-3]. Thirty-one percent of patients with multiple trauma are at risk for abdominal injuries and 31% of these patients experience liver injury [4].

An elevation in liver enzymes is observed in trauma patients, which indicates damage to liver tissue [5].

In addition to imaging tests and physical examinations, biochemistry tests are also used in the evaluation of trauma patients [6]. Aminotransferases, including aspartate transaminase (AST) and alanine transaminase (ALT), are indicators of liver cell injury and are used in diagnosis of acute liver diseases such as acute hepatitis. AST is found in the liver, heart muscle, skeletal muscles, kidneys, brain, pancreas, lungs, leukocytes and red blood cells, in order from high to low concentrations, while ALT exists primarily in the liver and is considered as a specific indicator for liver injury. Low concentrations of aminotransferases are found in serum in normal conditions but following hepatic trauma and injury to hepatocyte membrane, these enzymes are released into the blood circulation and their blood concentration is significantly increased [7, 8]. The normal range of aminotransferases varies among different laboratories but generally is considered 10-40 IU/l [9, 10]. Thus, the changes in concentration of aminotransferases could be useful for diagnosis of various pathologic conditions [10, 11]. It has been observed that reactive oxygen species (ROS), such as hydrogen superoxide (H_2O_2), increase in liver ischemic injury [12]. The formation of ROS and oxidative stress are the main mechanisms of the disease in the majority of ischemic liver injuries [13].

Extracts of *Silybum marianum* leaves and fruits have been used for centuries to treat liver, spleen and gallbladder disorders. In the 1960s, the active ingredient in extracts of seeds and fruits was isolated and its chemical structure was clarified. This substance was called silymarin, which is a mixture of flavonolignans. Flavonoids are natural substances that have various medicinal and therapeutic properties. Some of them have an antioxidant effect due to their phenolic structures and inhibit free radical-induced processes [14, 15].

Silymarin possesses several properties, including antioxidant, anti-inflammatory, protein synthesis stimulator, hepatoprotective, anti-fibrotic, anti-cancer, anti-cell proliferation, immune response modulator and anti-viral. Silymarin is also capable of reduction or prevention of the toxic effects of drugs such as cisplatin, vincristine, acetaminophen and gentamicin on the kidneys [16-19]. The most important antioxidant mechanism of silymarin include prevention of free radical formation through inhibition of the enzymes involved in the production of ROS, direct destruction of free radicals, chelation of heavy metals in the intestine, stimulation of the synthesis of protective molecules against stressful stimuli, such as thioredoxin, sirtuins, activation of antioxidant enzymes such as superoxide dismutase, and stimulation of protein synthesis through hepatocyte survival [20-22].

This study was conducted to investigate the positive effect of silymarin on liver enzymes and antioxidant status in trauma patients with elevated liver enzymes admitted to the intensive care unit (ICU).

Material and methods

Study design and setting

This randomized, double-blinded, placebo-controlled clinical trial was conducted in two ICUs of Shahid Rajaee Hospital, a teaching center affiliated to Shiraz University of Medical Sciences (SUMS), from August 2019 to August 2020.

Ethical approval

This study was approved by the Ethics Committee of the SUMS (IR.SUMS.REC.1398.768) and was registered in the Iranian Registry of Clinical Trials (IRCT20190911044744N1). Written informed consent was obtained from patients if conscious or their relatives. The trial was in accordance with the guidelines laid down in the 1975 Helsinki Declaration as revised in 2008.

Study population

All trauma patients with elevated liver enzymes (AST or ALT more than 2 times the upper limit normal) admitted in the ICU were included in the study if they met the following criteria: age of 16 or older, not having received a known hepatotoxic drug (ketoconazole, tetracyclines, isoniazid, rifampin, amoxicillin or clavulanate, macrolides, trimethoprim sulfamethoxazole, nitrofurantoin, clindamycin, phenytoin, valproic acid, carbamazepine, phenobarbital, acetaminophen, amiodarone, statins and propofol) [23-25], no history of oral silymarin administration over the past 7 days, not receiving concurrent medications with antioxidant properties, such as N-acetyl cysteine, vitamin C, E and A, willingness to participate in the study and no confirmed history of allergic reactions to silymarin. The patients were excluded from the study if they were pregnant or lactating or if they had a history of liver disease.

The patients were assigned to placebo or treatment groups using block randomization. Subjects in the treatment and placebo group received Livergol tablets containing 140 mg of silymarin three times daily (Goldaru Herbal Products Pharmaceutical Company, Isfahan, Iran) and 140 mg of placebo manufactured by the faculty of pharmacy of SUMS and similar to Liver-

gol tablets in size, shape, weight, color, and taste three times daily, respectively.

Measurements and study outcomes

The patients' information including demographic data, laboratory profiles, disease history and medications was recorded through reviewing the medical charts of the patients, as well as interviews with the patients or their relatives. Also, the Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) II score on admission was recorded for each patient [26]. Blood samples (5 ml) were obtained at baseline; AST, ALT, alkaline phosphatase (ALP), direct bilirubin, albumin, prothrombin time (PT), serum electrolytes (potassium, sodium), blood urea nitrogen (BUN) and serum creatinine were all measured at baseline, as well as days 3, 7, 9 and 14 after Livergol or placebo administration. Also, prior to the intervention and at the end of the study (day 14), blood samples (5 ml) were collected from each patients and serum was separated by centrifugation at 2500 rpm for 10 min; following centrifugation, samples were stored at –80°C. At the end of the trial, the frozen blood samples were used for measuring total antioxidant capacity (TAC), malondialdehyde (MDA), and thiol groups. These measurements were performed using Zantox commercial kits (Kavosh Arian Azma Co, Iran).

For assessment of thiol groups, the Ellman's reagent method was used [27]. Based on this method, free thiol groups react with 2-2/-dinitro-5,5/-dithiodibenzoic acid and a yellow complex is produced. The obtained complex was measured spectrophotometrically at 412 nm (EPOCH plate reader, Highlandpark, USA).

TAC was measured using the ferric reducing antioxidant power (FRAP) method [28], in which reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) by electron donating antioxidants produces a blue complex under acidic pH and in the presence of 2,4,6-tripyridyl-s-triazine (TPTZ) reagent. The absorbance of the blue complex was measured using a spectrophotometer at 593 nm.

MDA concentration was measured by the TBA reaction method [29]. Under acidic conditions and a temperature of 95°C, one molecule of MDA reacts with two molecules of TBA and this reaction produces a pink complex, which has maximum light absorption at a wavelength of 532 nm. The fluorescent intensity of the complex was measured at 553 nm with excitation at 515 nm using a spectrophotometer.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics Version 20 software (SPSS Inc./IBM Corp., Chicago, IL, USA). The analysis was conducted on data available to all subjects completing the study (per protocol analysis). The one-sample Kolmogorov-Smirnov test was performed to evaluate the normality of the distribution of the data. Categorical variables were presented as absolute and relative (percentage) frequencies. Continuous variables were expressed as mean ± standard deviation (SD).

The independent sample *t*-test was employed to compare parameters between silymarin and placebo groups. Also, the paired sample *t*-test was used for means comparison of variables in order to identify within-group differences. The repeated measure ANOVA test was applied to compare the changes in the investigated markers from baseline to 14 days follow-up between two groups. *P* values < 0.05 were considered statistically significant.

Results

The consort diagram of the study is shown in Figure 1. During the study period, 126 patients were screened for recruitment, of whom 107 subjects were included in the trial, 53 in the silymarin group and 52 in the placebo group. Finally, 90 patients completed the study, including 45 cases in the silymarin group and 45 patients in the placebo group.

The participants' demographic and relevant clinical information is shown in Table 1. There was no significant difference between the two groups in terms of the baseline characteristics and clinical findings, including sex, age, weight, APACHE II score, serum electrolytes, serum creatinine, BUN, INR, direct bilirubin and serum albumin ($p > 0.05$).

The level of liver enzymes before and on different days of the intervention are shown in Table 2. At baseline, there were no significant differences between the silymarin and placebo group in terms of ALT, AST and ALP. AST and ALP levels were not significantly different between the two groups on day 3 ($p = 0.43$ and $p = 0.75$, respectively) but they were significantly lower on days 7, 9 and 14 in silymarin treated patients ($p = 0.04$, $p < 0.001$ and $p < 0.001$ for AST and $p = 0.041$, $p = 0.027$ and $p < 0.001$ for ALP, respectively). ALT level was also significantly lower in the silymarin group on days 9 and 14, compared to the placebo group ($p < 0.001$ for both days), while no significant difference was found on days 3 and 7.

Moreover, the results from repeated measure analysis showed that AST, ALT and ALP levels significantly decreased ($p < 0.001$) in both silymarin and placebo groups. This decrease in liver enzymes was significantly higher in silymarin treated patients than the placebo group ($p < 0.001$). Also, no significant differences were observed in the mean change in AST, ALT, and ALP values at baseline, and days 3, 7, 9, and 14 in placebo and silymarin treated patients ($p > 0.05$).

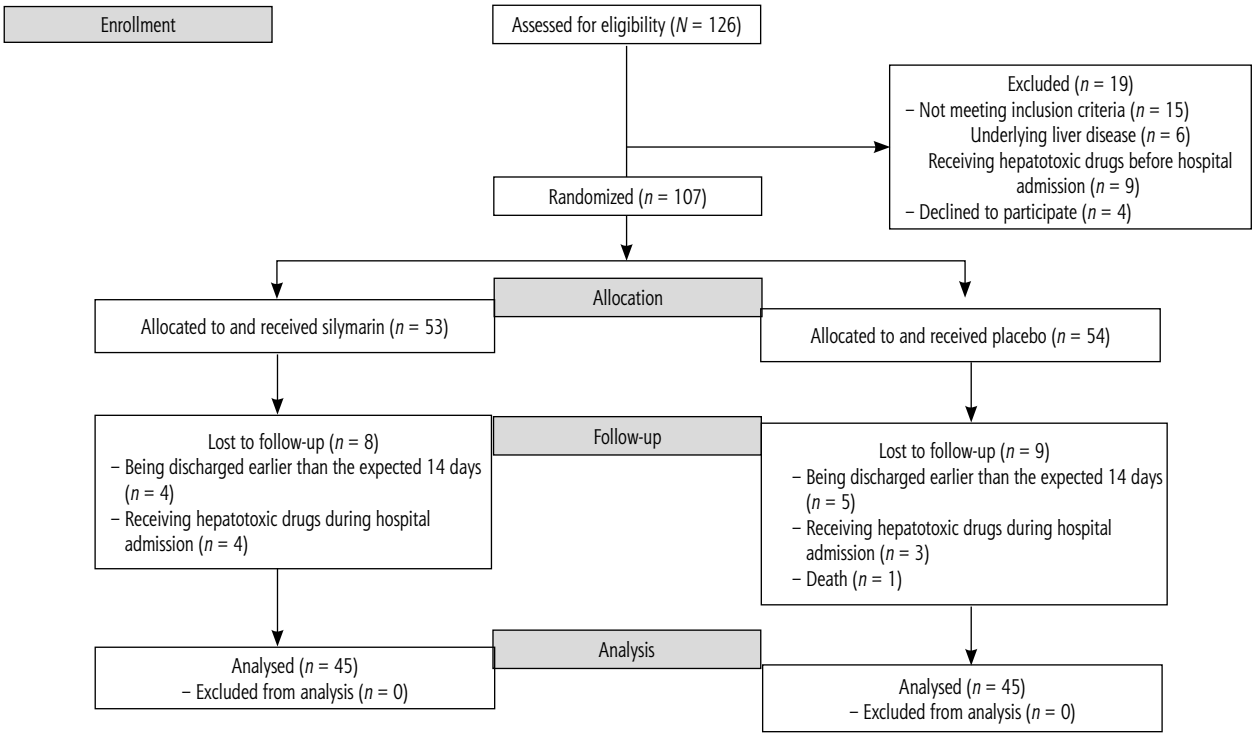

Fig. 1. Flowchart of the study

Regarding international normalized ratio (INR) and albumin values, no significant differences were observed between the study groups on days 3, 7, 9 and 14 (all $p > 0.05$).

Table 1. Demographic information and baseline laboratory findings in silymarin and placebo groups

Variables	Mean ± SD or *n* (%)		*P*-value
	Silymarin group	Placebo group	
Sex			
Male	25 (55.6)	21 (46.7)	0.52
Female	20 (44.4)	24 (53.3)	
Age (years)	52.02 ±14.92	54.15 ±17.38	0.53
Weight (kg)	65.66 ±12.67	67.22 ±10.59	0.53
APACHE II score	14.48 ±4.92	14.60 ±4.64	0.91
Mg (mg/dl)	1.59 ±0.42	1.61 ±0.49	0.85
Na (mEq/l)	138.37 ±2.41	138.84 ±2.90	0.41
K (mEq/l)	4.30 ±0.67	4.27 ±0.50	0.80
Ca (mg/l)	8.63 ±0.48	8.58 ±0.57	0.60
Albumin (g/dl)	3.34 ±0.49	3.26 ±0.65	0.50
Direct bilirubin (mg/dl)	0.4 ±0.21	0.43 ±0.19	0.54
INR	1.21 ±0.21	1.22 ±0.21	0.72
Serum creatinine	1.47 ±81	1.42 ±67	0.76
BUN	21.02 ±7.47	21.08 ±5.90	0.96

SD – standard deviation, APACHE II score – Acute Physiology and Chronic Health Evaluation II Score, INR – international normalized ratio, BUN – blood urea nitrogen

With respect to antioxidant indices, including MDA, TAC and thiol groups, there were no significant differences between groups at baseline (Table 3). MDA level on day 14 was marginally significantly different between the groups ($p = 0.051$). Also, TAC and thiol groups were significantly higher in silymarin treated patients (both $p < 0.001$). In the silymarin group, MDA decreased significantly at the end of the treatment period compared to baseline ($p < 0.001$), while both TAC and thiol groups significantly increased (both $p < 0.001$). In the placebo group, MDA and thiol groups significantly changed on day 14, compared to the baseline. TAC decreased over time in the placebo group; however, this change was not statistically significant ($p = 0.9$).

The mean length of hospital stay was not significantly different between silymarin and placebo groups (23.8 ±5.11 vs. 24.2 ±3.81, $p = 0.64$). No adverse effects associated with silymarin were observed and there were no reports of gastrointestinal symptoms. All subjects tolerated silymarin well. No mortality was found in the silymarin group, while one patient died in the placebo group.

Discussion

This study was conducted to evaluate the efficacy of silymarin supplementation on liver injury induced by trauma and its related markers. To the best of our knowledge, this clinical trial is the first human study testing the effects of silymarin on serum levels of anti-

Table 2. Comparison between mean aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase on different days in silymarin and placebo groups

Variables	Day	Silymarin	Placebo	*P*-value
AST (U/l)	Baseline	215.14 ±91.61	201.88 ±65.78	0.43
	Day 3	180.42 ±85.22	197.48 ±58.31	0.27
	Day 7	144.24 ±73.37	184.93 ±57.02	0.04
	Day 9	111.33 ±61.43	161.37 ±55.01	< 0.001
	Day 14	79.26 ±49.95	133.97 ±52.94	< 0.001
ALT (U/l)	Baseline	181.84 ±81.59	178.22 ±65.78	0.81
	Day 3	149.15 ±74.19	176.04 ±55.50	0.055
	Day 7	116.55 ±63.39	164.82 ±55.19	0.062
	Day 9	84.86 ±52.28	138.73 ±53.63	< 0.001
	Day 14	58.75 ±42.10	112.75 ±51.39	< 0.001
ALP (U/l)	Baseline	272.15 ±44.06	268.05 ±54.11	0.79
	Day 3	254.15 ±42.09	258.70 ±50.77	0.75
	Day 7	222.40 ±39.19	252.30 ±49.68	0.041
	Day 9	197.60 ±39.42	229.07 ±50.80	0.027
	Day 14	154.80 ±40.11	209.45 ±49.69	< 0.001

AST – aspartate aminotransferase, ALT – alanine transaminase, ALP – alkaline phosphatase

Table 3. Comparison between mean malondialdehyde, thiol groups and total antioxidant capacity at baseline and at the end of the study in silymarin and placebo groups

Variables	Day	Silymarin	Placebo	*P*-value
MDA	Baseline	0.76 ±0.72	0.76 ±0.95	0.80
	Day 14	0.54 ±0.10	0.58 ±0.008	0.051
	P-value	< 0.001	< 0.001	
Thiol	Baseline	385.91 ±157.37	379.80 ±17.44	0.08
	Day 14	416.01 ±17.82	365.15 ±17.52	< 0.001
	P-value	< 0.001	< 0.001	
TAC	Baseline	798.92 ±17.13	802.96 ±13.61	0.21
	Day 14	878.78 ±27.84	802.83 ±14.58	< 0.001
	P-value	< 0.001	0.9	

MDA – malondialdehyde, TAC – total antioxidant capacity

oxidant markers and liver enzymes in trauma-induced liver injury.

The primary findings of this study suggested that receiving 140 mg of silymarin supplement three times daily for 14 days could significantly lower the liver enzymes, including ALT, AST and ALP, compared to placebo.

Several beneficial effects of silymarin have been identified, including antioxidant, anti-inflammatory, hepatoprotective and anti-fibrotic properties, as well as modulation of insulin resistance [30, 31]. In line with our results, the findings of two animal studies indicated the protective effects of silymarin against liver injury. Kim *et al*. reported that oral administration of silymarin in mice can prevent stress-induced liver damage through its antioxidant and anti-inflammatory activities. Significantly higher serum levels of ALT and AST were observed in the control group in this study. Also, Yamisen *et al*. evaluated the positive effects of silymarin in burn-induced liver injury in burned rats and observed that both topical and systemic treatments with silymarin were effective in reversing this type of liver injury [32].

Several clinical trials have been conducted on the hepatoprotective effects of silymarin in different pathologic situations. Luangchosiri *et al*. reported that patients receiving 140 mg of silymarin three times daily were at lower risk for antituberculosis drug-induced liv-

er injury. However, the median changes of serum transaminase, glutathione and MDA were not significantly different between the silymarin and control group [33].

In contrast to our results, another randomized trial evaluating the effect of silymarin in patients with acute clinical hepatitis with any etiology reported no detectable effect on biomarkers of the hepatocellular inflammatory process, including ALT and AST. However, silymarin significantly improved subjective symptoms and signs related to biliary retention [34].

As mentioned above, considering the potential anti-inflammatory and antioxidant effects of silymarin, as well as its free radical scavenging activities, it has been proposed that it can be beneficial in management of pathological conditions associated with oxidative stress and free radicals, such as diabetes, a wide range of cancers, alcoholic liver diseases, liver cirrhosis, *Amanita* mushroom poisoning, viral hepatitis, toxic and drug-induced liver diseases, drug-induced kidney injury and end stage renal disease [35-39]. However, no studies have been conducted on the effect of silymarin on trauma-induced liver injury. Trauma can interrupt the blood supply to the liver and consequently cause hepatic ischemia/reperfusion injury. During the reperfusion phase, the release of ROS activates Kupffer cells, and stimulates inflammatory cytokine production, as well as infiltration of leucocytes, which in turn induces damage to liver cells [40]. Thus, this evidence supports the use of silymarin in trauma-induced liver injury.

In this manner, our results showed that the mean serum level of MDA was significantly decreased and the mean serum levels of TAC and thiol groups were significantly increased in the silymarin group from baseline to day 14. In the placebo group, mean serum levels of MDA and thiol groups were significantly increased, while serum level of TAC was not significantly changed on day 14, compared to baseline. Comparing placebo and treatment groups revealed that the mean serum level of MDA was significantly lower, while the serum levels of thiol groups and TAC were significantly higher in the silymarin group. Ebrahimpour *et al.* in a clinical trial evaluating the effect of silymarin supplementation on antioxidant status in patients with type 2 diabetes mellitus reported that TAC, superoxide dismutase, and glutathione peroxidase activity levels were significantly higher in the treatment group compared to the placebo group. Also, MDA was significantly decreased in the silymarin group compared to the baseline value [39]. Our results were in agreement with these results.

The most important side effects of silymarin are gastrointestinal symptoms including diarrhea, dyspepsia, irregular stools and nausea [41]. All the patients treated with silymarin tolerated it well and no adverse effects were observed among the participants. Our results are in line with previous studies, which confirmed the safety of silymarin [19, 42].

Our study had some limitations which should be addressed. First, the sample size in this study was relatively small and the intervention period was short, limited to two weeks ($\alpha = 5\%$ and $\beta = 10\%$, with a statistical power of 80%). Second, due to limitations, the antioxidant status markers were measured only at two time points and the daily change trend was determined. Another limitation was the possibility of gradual degradation of antioxidant markers in serum samples during storage in the freezer at –80°C for a relatively long period of time (up to one year for the first collected samples). A further limitation is using the conventional form of silymarin, which is associated with lower bioavailability, more extensive metabolism and lower permeability through the intestine, compared to modified formulations.

Conclusions

In conclusion, our results showed that supplementation with 140 mg of silymarin three times daily could significantly lower the serum levels of AST, ALT and ALP in trauma patients with increased levels of liver enzymes. Also, a significant reduction in MDA level and an increase in TAC and thiol groups were observed in the silymarin treated group. Silymarin could be a helpful complementary treatment in trauma-induced liver injury. Further studies with larger sample sizes and longer follow-up durations are required to better determine the efficacy of this treatment modality.

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Disclosure

The authors declare no conflict of interest.

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