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Effects of lactulose and silymarin on liver enzymes in cirrhotic rats.

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ABSTRACT

Silymarin (SM), a mixture of anti-hepatotoxic flavonolignans is being used for liver diseases and lactulose (LAC), a non-absorbable synthetic disaccharide were used in this study to analyze their probable synergic effects on hepatic cirrhotic rat model to see whether they cause improvement of this illness or vice versa. Upon thioacetamide(TAA) withdrawal and treatment administration. The curative effects of SM and LAC were confirmed by significant decrease of liver enzymes and malondialdehyde (MDA) levels. In SM+LAC group, the liver enzymes and malondialdehyde levels were decreased significantly compared with TAA group. All treatments were able to regenerate and trigger an enhanced regeneration. Silymarin and lactulose alone or in combination had potent curative effects and reduced thioacetamide effects induced liver damage.

Keywords: Liver Cirrhosis; Rat; Silymarin; Thioacetamide.

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Introduction	52
Cirrhosis is a result of chronic liver injury defined by development of regenerative nodules	53
bordered by fibrous bands that leads to portal hypertension and end-stage liver disease	54
(Schuppan and Afdhal 2008) and in some patients to formation of portosystemic collateral	55
veins which in high blood ammonia concentrations leads to portosystemic encephalopathy	56
(Tarantino et al. 2009). Liver transplantation remains the only beneficial option for some	57
patients, but pharmacological treatments that can stop progression or even reverse cirrhosis	58
are currently being developed (Schuppan and Afdhal 2008).	59
Thioacetamide induces hepatotoxicity, originally used as fungicide. Earlier literature suggests	60
that an obligate intermediate metabolite of thioacetamide which binds to proteins, forming	61
acetylimiodolysine derivatives, is responsible for TAA-induced hepatotoxic effects (Kumar	62
et al. 2014).	63
At present, one of the therapeutic strategies for liver fibrosis includes complementary and	64
alternative medicine. Animal and clinical evidence have confirmed that any degree of fibrosis	65
and even cirrhosis are potentially reversible by reasonable therapeutic strategies (Zhou et al.	66
2014).	67
We shouldn't consider cirrhosis as the irreversible end stage of chronic liver disease, but	68
rather as a stage in an active process whose advance course(progression or reversion of	69
fibrosis) depends on the providing of proper treatment and the avoidance of further harmful	70
effects. Appropriate treatment can stabilize cirrhosis or even reverse it (Wiegand and Berg	71
2013).	72
Milk thistle (<i>Silybum marianum</i>) has been the dietary supplement taken more frequently by	73
patients with chronic liver diseases. As a result, this plant is now being more widely used in	74

the treatment of liver diseases such as toxic hepatitis, fatty liver, ischemic injury, cirrhosis, 75
viral hepatitis and radiation toxicity (Luper 1998). Both milk thistle and its active ingredient 76
silymarin are pharmacologically safe and well tolerated (Feher and Lengyel 2012). Silymarin 77
is a lipophilic extract of the milk thistle seeds containing various flavonolignans (which 78
silybin is the major one), has received a tremendous attention over the last decade as an 79
herbal remedy for liver treatment over 30 years. In many cases, the antioxidant properties of 80
silymarin are considered to be responsible for its protective actions (Surai 2015), through 81
alleviating oxidative stress and inflammatory response (Feng et al. 2016). Silymarin is 82
commonly prescribed in cases of viral hepatitis or cirrhosis (Del Prete et al. 2012) because it 83
has hepatoprotective activity (Al-Sayed and Esmat 2016). 84

Lactulose modifies the bacterial profile in intestinal contents, increasing the lactobacilli and 85
bifidobacteria counts, up-regulating short-chain fatty acids production (Algieri et al. 2014). 86

Lactulose used for the treatment of hepatic encephalopathy(HE) and is effective for primary 87
prevention of overt hepatic encephalopathy in patients with cirrhosis (Sharma et al. 2012). 88

Lactulose decreases the blood endotoxin level and reduce liver damage. It is suggested that 89
lactulose can prevent absorption of endotoxin from the gut and may have an effect on gut- 90
derived endotoxemia (Hou 1991). 91

Liver regeneration evolved presumably to protect wild animals from catastrophic liver loss 92
caused by toxins or tissue injury (Wu et al. 2015). Lactulose administration accelerates post 93
hepatectomized liver regeneration in rats by inducing hydrogen, which may result from 94
attenuation of the oxidative stress response and excessive inflammatory response (Yu et al. 95
2015). Long-term administration of silymarin significantly increased the survival time of 96
cirrhotic alcoholic patients (Feher and Lengyel 2012). But in case of silymarin, Wu and Tsai 97
et al. have stated that silymarin plays a crucial role in accelerated liver regeneration after 98
Partial hepatectomy. It accelerates the cell cycle in the G1 to S phases (Wu et al. 2015). In the 99

same time Cetinkunar and Tokgoz et al. demonstrated that silymarin does not contributed to
proliferative regeneration of the liver-which has very important metabolic functions -after
partial hepatectomy; instead it will decrease serum levels of *transaminases* (Cetinkunar et al.
2015).
Therefore, for clarifying this confliction we designed present study aimed to investigate the
potential curative effect of silymarin, lactulose or a combination of both against TAA-
induced liver cirrhosis in rats.

Materials and Methods*Materials, Drugs and chemicals*

Formaldehyde solution was purchased from (Dr. Mojallali Industrial chemical complex Co.,
Iran). Lactulose was purchased from (Alborz Daru, Iran), ketamine %10 and xylazine %2
were purchased from (alfasa, Holland), silymarin from the fruit of *Silybum marianum* extract
was purchased from (SIGMA-ALDRICH, USA), and thioacetamide, hydrochloric acid
fuming and 2- thiobarbituric acid were purchased from (Merck, Germany) and trichloroacetic
acid was purchased from (Central drug house (p) Ltd., India).

Animals

All the animal experiments were conducted in accordance the Guide for the Care and Use of
Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave.
NW, Washington, DC 20055, USA) and approved by the Bu- Ali Sina research committee.
Forty Wistar albino male rats weighting 210-275 g were used (Pasture Institute, Karaj, Iran).
The animals were kept in an agreeable environment in transparent plastic cages under
standard laboratory conditions (light period 7.00 a.m. to 7.00 p.m., 22±2 ° C, and humidity,
55±5%) with free access to food and water, they were acclimatized for at least 2 weeks
before the start of the study. The experiment lasted for 26 weeks.

Induction of liver cirrhosis

Liver cirrhosis was induced according to the method of Li et al. (Li et al. 2002) and Laleman et al. (Laleman et al. 2006). Thirty-two male Wistar rats were administered by TAA with drinking water for 18 consecutive weeks (124 days). Eight rats, drinking natural water, were randomly chosen as the healthy controls. In the 12th week, three rats were sacrificed to monitor the development of cirrhosis (Figure 5.). In cirrhotic rats only 2 rats were died. After 18 weeks TAA water was withdrawn and replaced by tap water or treatment according to rat groups.

Preparation of silymarin

Experimental diet was prepared by 50 mg/kg/day silymarin (Alshawsh et al. 2011), mixed into their chow before forming it into pellets weekly for 8 weeks (Toyoda-Hokaiwado et al. 2011).

Experimental design

Rats were divided randomly into five groups: (Table 1) Control group, TAA group: TAA-induced cirrhotic group, Silymarin (SM) group: hepatic cirrhosis treated with silymarin (50 mg/kg/day in diet), Lactulose group: hepatic cirrhosis treated with lactulose (2 g/kg/day in drinking water), SM + LAC group: hepatic cirrhosis treated with silymarin (50 mg/kg/day in diet) and lactulose (2 g/kg/day in drinking water) for 8 weeks.(Figure 1.)

Endpoints to evaluate hepatic injury and regeneration

The following endpoints were evaluated: (1). Liver injury: Serum *alanine amino transaminase (ALT) = Glutamic Pyruvic Transaminase (SGPT)*, *aspartate amino transferase*

(AST) = Glutamic Oxal acetic Transaminase (SGOT) and alkaline phosphatase (ALP). (2). 150
Liver regeneration: Calculating liver index (LI) according to the following formula: liver 151
index = liver weight (g) / bodyweight (g) × 100%.; (3). Oxidative stress: Malondialdehyde 152
(MDA) (Amin et al. 2012; Gu et al. 2011a; Gu et al. 2011b) . 153

Collection of blood samples and liver tissues 154 155

At the end of the experiment, all rats were fasted for 12 h then 5-8 ml whole blood samples 156
were taken directly from the heart under ketamine 10% - xylazine 2% anesthesia. The blood 157
samples were centrifuged at 10000 rpm for 20 min for serum separation. All animals were 158
sacrificed and their liver dissected out. Right lobe of the liver was cut and fixed in 10% 159
formalin for histopathological examination. 160

Biochemical analysis 161 162

Biochemical analysis in serum were done for determination ALT, AST and ALP, serum total 163
bilirubin, total protein (Pro), albumin (ALB) and gamma glutamyl transferase (GGT) levels 164
(Roche Hitachi 912 Chemistry Analyzer, Japan) using commercial kits (Man, Iran) for total 165
protein (Parsazmun, Iran) for others to evaluate the liver injury degree. 166

Preparation of liver homogenates 167 168

At the end of the experiment, all of the rats were decapitated and the liver was rapidly 169
isolated and weighted. This tissue was subsequently homogenized in cold 0.9% normal 170
saline. The homogenate was kept for lipid peroxidation assay. 171

Measurement of malondialdehyde 172 173

MDA measurement has been used frequently to measure lipid peroxidation. The lipid peroxidation assay was performed by determining the MDA, which is the end product of lipid peroxidation, and reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex that has a peak absorbance (A) at 535 nm. A mixture of trichloroacetic acid (TCA), TBA, and HCl were added to 1mL of serum, and the mixture was heated for 45 min in a boiling water bath. After cooling and centrifuging it at 1000 g for 10 min, the cleared supernatant absorbance was measured using SECOMAM S.750 Spectrophotometer at 535 nm. The level of TBARS was calculated by: $C (M) = A / 1.65 \times 10^5$ (Rajaei et al. 2013).

Histopathological examination

Formalin 10% fixed liver tissues were embedded in paraffin, and then they were cut into 5 μ m thick sections. The slides were stained with the conventional hematoxylin and eosin (H&E) and masson's trichrome protocol, mounted with neutral resin, and examined with optical microscopy.

Assessment of necroinflammatory activity grades and staging:

Liver samples stained with H&E and trichrome masson were assessed for total and individual activity parameters guided by ishak's histological activity index (Ishak et al. 1995). Classified according to the following parameters: confluent necrosis (0-6), periportal or periseptal interface hepatitis (piecemeal necrosis) (0-4), portal inflammation (0-4), focal (spotty) lytic necrosis, apoptosis and focal inflammation (0-4), and architectural changes, fibrosis and cirrhosis (0-6). Additional features which were noted but not scored: bile-duct inflammation, damage and proliferation and steatosis, mild, moderate or marked.

<i>Statistical analysis</i>	199
All data were presented as mean ± SD. The analysis was carried out using Statistical Package	200
for the Social Sciences (SPSS) version 22. Statistical analyses were conducted by One-way	201
Analysis of Variance (ANOVA) and followed by Least Significant Difference (LSD)	202
analyses to compare various groups with each other. Statistical analyses for scoring of	203
histological parameters were done by Mann- Whitney <i>U</i> from two independent sample tests.	204
Significant differences were analyzed at <i>P</i> < 0.05.	205
	206
Results	207
<i>Mortalities and liver function</i>	208
During the whole experimental period, there were minor rat deaths. A total of three rats died:	209
one rat died from the normal group; two rats died in making cirrhotic model. The mortality	210
rate was not more than 7.5% which makes the survival rate of the model 92.5%. (Table 1).	211
Levels of serum <i>ALT</i> and <i>AST</i> were assayed as markers of liver injury. thioacetamide	212
produced liver cirrhosis characterized by a significant increase in serum <i>ALT</i> and <i>AST</i> levels	213
compared with control group. Although thioacetamide have been withdrawn for 8 weeks but	214
in TAA group serum <i>ALT</i> and <i>AST</i> levels was high and significant in <i>AST</i> comparing with	215
control group (<i>P</i> < 0.05), while treatment with treatments, decreased <i>AST</i> and <i>ALT</i> levels.	216
Silymarin administration decreased <i>AST</i> and significantly decreased <i>ALT</i> level compared with	217
a TAA group (<i>P</i> < 0.05). Lactulose and both silymarin and lactulose significantly decreased	218
liver enzymes (<i>P</i> < 0.01 and <i>P</i> < 0.05) as shown in (Figure 2, A and B).	219
The plasma <i>ALP</i> level of the thioacetamide group increased significantly (<i>P</i> < 0.001) when	220
compared with the control group (Figure 2C). <i>ALP</i> levels was significantly decreased in SM,	221
LAC and SM+LAC groups by comparing with TAA group.	222

Liver dysfunction improved upon thioacetamide withdrawal and treatment administration, with decrease of *ALT*, *AST* and *ALP* it almost resume to the levels as that of the healthy rats (Figure 2A, B and C). In the silymarin group, liver dysfunction improved upon thioacetamide withdrawal with decrease of *ALT*, *AST* and *ALP* and resume to the levels lower as that of the healthy rats in *ALT*. In lactulose group, recovery of liver dysfunction noted to improve after thioacetamide withdrawal as compared with the controls and with the decrease of *ALT* and *AST* resume to the levels lower as that of control rats but *ALP* did not resume to the levels as that of control rats. In SM + LAC group, recovery of liver dysfunction resume to the levels of control rats in *AST* and lower of control rats in *ALT* but still *ALP* is higher than control group. There was a significant increase ($P < 0.05$ and $P < 0.01$) in serum total bilirubin of lactulose group (2 g/kg/day) when compared with control, TAA and SM groups. Therefore there are no significant differences between TAA, SM and SM + LAC groups and the control group, as shown in (Table 2).

It seems that after 8 weeks liver dysfunction improved upon thioacetamide withdrawal and liver ability to synthesize the *albumin* and other proteins resume to the levels as that of the healthy rats so there are no significant differences between all groups and the control group, except for SM+LAC which are significantly lower than TAA group as shown in (Table 2) which it could be explained by *albumin*'s long half-life.

Table 3 indicates the effect of silymarin and lactulose and their combination on the *GGT* levels in the serum of control and experimental groups of rats. This marker enzyme is significantly ($P < 0.05$) increased in TAA and SM + LAC animals when compared with control animals. (Table 2)

Effect of silymarin and lactulose on lipid peroxidation

MDA was measured to evaluate oxidative stress. MDA, as an oxidation product of lipid peroxidation injury, increased non-significantly in serum after exposure to thioacetamide (Figure 3). Treating with lactulose or silymarin or their combination non-significantly lowered MDA changes, respectively (Figure 3).

Effects of silymarin and lactulose and their combination on the liver index

After 18-weeks of thioacetamid administration and 8-weeks of treatment, the weight of rats in the TAA group was non-significantly less than rats in control group (Table 3). The weight of rats in LAC and SM+LAC groups non-significantly increased compared with control and TAA groups. Weight of liver in LAC and SM + LAC groups were significantly higher than liver weight in control group ($P < 0.05$). The liver index used to assess liver damage. LIs of LAC and SM+LAC rats increased and remained at significant higher levels to that of the control ($P < 0.05$), except for SM group which wasn't remarkably higher than control group (Figure 4). Liver index in the thioacetamide group increased compared with those of normal group, while silymarin group was decreased compared with thioacetamide group but wasn't significant. The results showed that hepatomegaly alleviated after the treatments (Figure 4).

Making cirrhotic model

Thioacetamide-induced cirrhosis was established at week 18 after administration thioacetamide, confirmed both macro and microscopically (Figure 5.). Thioacetamide increased amount of collagen deposition in liver tissue stained with masson's trichrome significantly.

Effect of silymarin and lactulose on hepatic cirrhosis

The liver tissue has examined by light microscope for histopathological studies. For determination of liver injury histological examinations have done on sections stained with H&E and masson stain. For clarifying the extent of hepatic cirrhosis induced by thioacetamide, masson's trichrome had used for liver sections staining. The amount of collagen deposition has increased significantly by thioacetamide in liver tissue, which stained with masson trichrome. Cirrhotic pattern of affected liver was micro nodular, macro nodular, abnormal polyhedron architecture collagen fiber hyperplasia and liver cell necrosis in portal area that extended into the surrounding liver lobules, this leads to criteria of hepatic cirrhosis. In control group of rats we observed normal architecture of liver cells, containing uniform granulated cytoplasm and small nuclei. Collagen fibers are thin and delicate (Figure 5).

Effect of silymarin and lactulose and their combination on hepatic necroinflammatory activity

Examination of TAA group showed moderate to severe necroinflammatory activity with average score of (8.86) with marked confluent necrosis and moderate apoptosis and focal inflammation with mild portal inflammation. Treatment with silymarin and lactulose alone or in combination healed the liver from hepatotoxic effects of thioacetamide reflected by increase of necroinflammation to (9.29), (9.71) and (9.71), respectively but there wasn't any significant difference between SM, LAC and SM+LAC groups and TAA group as shown in (Figure 5 and 6).

DISCUSSION

Key to the first step was to making a cirrhotic animal model. Prolonged exposure to thioacetamide has been reported to result in liver cirrhosis. The toxin affects both perivenular and periportal areas. This model develops macro nodular cirrhosis with portal hypertension,

the instauration of overt hyper dynamic circulation, associated with the typical histological, 298
biochemical and hemodynamic changes observed in man (Laleman et al. 2006), fibrosis 299
remains stable for weeks after thioacetamide withdrawal (Abralles et al. 2006). In this study, 300
cirrhotic rat model was successfully developed via drinking thioacetamide water for 18 weeks 301
and was confirmed by histological evidence. 302

Analysis of histopathology (H&E staining and masson staining) showed that structure of rat 303
liver in the control group was integrity, the central vein was visible, liver cells were arranged 304
radially and neatly, and lobular contour was clear. However, in liver cirrhosis group the 305
structure of rat liver was abnormal, hepatic lobular architecture was destroyed, pseudo lobule 306
is formed, collagen deposition was around the portal region and connects to each other to 307
form nodules and distort the architecture, and extensive fibrosis with broad septa was 308
observed (Figure 5). These results definitely indicated that the rat model of liver cirrhosis was 309
successfully established. 310

Thioacetamide-induced hepatotoxic effects characterized by confluent necrosis, piecemeal 311
necrosis, apoptosis and focal inflammation and focal (spotty) lytic necrosis on tissue levels 312
(Figure 5) which reflected in our study by significant elevation of serum liver enzymes 313
(Figure 2) and *GGT* (Table 2) as well as increased collagen deposition and fibrotic areas 314
significantly. The statistically significant increase of the *GGT* concentrations is due to the 315
metabolic consequence of TAA induced dysmetabolism leading to severe hepatic steatosis, of 316
which *GGT* is a marker. These results are in accordance with many investigations on 317
thioacetamide - induced liver fibrosis and cirrhosis in rats (Gu et al. 2011a; Gu et al. 2011b; 318
Kawai et al. 2012; Laleman et al. 2006). 319

In order of interfere avoidance of thioacetamide with our observation endpoints, 320
thioacetamide has withdrawn before the treatment, which was based on the following 321
considerations. In the present study, the intention was to investigate regeneration of the 322

cirrhotic liver after treatment. The existence of thioacetamide, a hepatic toxin, would interfere, which would obscure treatment induced regeneration. In general, when used in combination with treatment, it would change treatments effect. Because the treatment period was as long as 56 days, thioacetamide continuously feeding up to 56 days would make most of the rats develop cholangiocarcinoma (Abralles et al. 2006; De Minicis et al. 2013; Moreira et al. 1995) or die of thioacetamide toxicity. Upon thioacetamide withdrawal, hepatic cirrhosis continuously resolve, but persisted up to 120 days, and liver regeneration significantly decelerates (Gu et al. 2011b). Hence, we have enough time to make treatment on cirrhotic rats. In the literature, withdrawal of thioacetamide prior to the study was also acceptable as in Prasad study on cirrhotic rats (Prasad et al. 2010).

Thioacetamide had been confirmed to induce hepatic injury and trigger proliferation of liver, noticeably increased the ratio of liver to overall body weight as presented in LI, which used to scrutinize potential changes in the liver size, in comparison with control rats (Figure 4).

Thioacetamide, a potent centrilobular hepatotoxicant, undergoes a two-step bio activation mediated by microsomal CYP2E1 to thioacetamide sulfoxide (TASO), and further to thioacetamide -S, S-dioxide (TASO₂), a reactive metabolite that initiates cellular necrosis (Chilakapati et al. 2005). Thioacetamide provokes a remarkable increase in the activity and amount of total mRNA, as well as in the size of the nucleoli of liver cells. During the biotransformation of thioacetamide both cytochrome P450 and *flavin-containing monooxygenase (FMO)* reduce dioxygen to superoxide anion, which is catalyzed to form hydrogen peroxide. Therefore, biotransformation of thioacetamide precedes oxidative damage associated liver injury. This had been already implicated in the disappearance of tetraploid hepatocytes and the presence of an increase in MDA in liver cells following thioacetamide administration (Constantinou et al. 2007). By considering the role of oxidative stress in disease, malondialdehyde assay is the most generally used test. Malondialdehyde is

one of the several products formed throughout polyunsaturated fatty acids breakdown 348
induced by radicals (Lefevre et al. 1997). Silymarin and lactulose alone or in combination 349
protected the liver from oxidative stress caused by thioacetamide as they decreased 350
significantly the elevated serum MDA (Figure 3). In this study, oxidative stress after cirrhosis 351
manifested as increased lipid peroxidation. After silymarin treatment silymarin acts as a 352
scavenger of free radicals, so it decreases oxidative stress. The antioxidative property of 353
hydrogen might play a main role in the molecular mechanism of lactulose-enhanced liver 354
regeneration. 355

GGT is one of the important markers of liver injury, and their elevation discloses cholestasis 356
and bile duct necrosis. When liver cells are damaged, the serum *AST* and *ALT* are released 357
into the blood from the cells. Therefore, *ALT* and *AST* are two of the most reliable markers of 358
necrosis or hepatocellular injury. Data from the present study have demonstrated that SM and 359
LAC and SM+LAC groups protected liver from tissue inflammation, which exhibited an 360
obvious decrease in serum markers of liver function brought back these enzymes to near 361
routine level by possibly preserving the functional integrity of the hepatocytes, showing their 362
defense action against thioacetamide -induced hepatotoxicity as compared to the model 363
control (TAA group) (Figure 2). Silymarin decreased serum *GGT* level but it didn't happen in 364
LAC and SM+LAC groups (Table 3). Treatments decreased fibrosis scores in LAC and 365
SM+LAC groups compared to TAA group although it wasn't significant due to the short time 366
of observations. (Data not shown) (Figure 5) indicate effectiveness of silymarin, lactulose or 367
both in the recovery of liver function after cellular damage. Amin et al. and Fadillioglu et al., 368
respectively (Amin et al. 2012; Fadillioglu et al. 2010) demonstrated that silymarin and 369
lactulose significantly reduced the increased *ALT* and *AST* caused by thioacetamide. These 370
are evidence for the important role of silymarin and lactulose alone or in combination in 371
protecting cells from lipid peroxidation and restoring cell membrane function so they have 372

hepatocurative effects. In accordance to these results, observations indicated that an increase in serum liver enzymes levels in TAA group may be result of massive necrosis and late stage of fibrosis (Gu et al. 2011a; Gu et al. 2011b). The elevation of the serum activities of *ALP* and *GGT* indicated the induction of cholestasis (Kawai et al. 2009) while decreased serum liver enzymes activities in SM and LAC groups may be due to recovery, as decreasing serum liver enzymes activities in hepatic injury occur with recovery, given that lactulose has a function of reducing blood ammonia levels, and hydrogen produced by anaerobic organisms was the key for its therapeutic effects.

We found that silymarin, lactulose and their combination have dramatic effects on MDA, as serum MDA significantly decreased. The probable antioxidant mechanisms of silymarin are direct scavenging free radicals and chelating free Fe and Cu, preventing free radical formation, improving the integrity of mitochondria's electron transport chain in stress conditions, maintaining an optimal redox balance in the cell, activating *vitagenes*, and providing additional protection in stress conditions, affecting the microenvironment of the gut, have hepatoprotective effects, decreasing inflammatory responses, is an emerging mechanism of silymarin protective effects in liver toxicity and various liver diseases (Surai 2015). The exposure of hepatocytes to free fatty acids in the context of dyslipidemia, resulting in increased ROS production and mitochondrial damage (Tarantino et al. 2010), rapid fermentation of lactulose into organic acids at rates exceeding colonic buffering capacity to reduce formation of toxic fatty acids and ammonia from amino acids, polypeptides, and blood in the colon maybe is the probable mechanism of lactulose (Bonnkn 1990).

CONCLUSIONS

In conclusion, our findings of this study are: (1) As proved by serum markers for liver function and histological findings, silymarin, lactulose and silymarin & lactulose combination could protect against thioacetamide-induced liver cirrhosis in rats. (2) Silymarin & lactulose combination should be useful for the treatment of liver cirrhosis at physiological concentrations. (3) Silymarin, lactulose, and silymarin & lactulose combination exert significant curative effects against thioacetamide -induced oxidative stress and liver damage by augmenting host antioxidant defense mechanisms. (4) Silymarin & lactulose combination curative effects are due to increase of antioxidative effects as well as lowering the extent of lipid peroxidation.

Declarations

Ethics approval and consent to participate

All the animal experiments were conducted in accordance with the guideline and approved by the Bu-Ali Sina research committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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	424
Authors' contributions	425
Mozhgan Ghobadi Pour: Done the entire project.	426
<u>Naser Mirazi</u> : Project supervisor and corresponding author.	427
Hojjatollah Alaei: Supervisor assistant, project done in Dr. Alaei's lab and he provide all	428
what was needed.	429
Shirin Moradkhani: Proofreading the article.	430
Ziba Rajaei: Supervised the MDA test.	431
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Table 1- Experiment design and mortalities.

Experiment groups	Mortalities	Subgroups(7 rats)	Dose
Healthy rats(8 rats)	1/8	Control	-
Cirrhotic rats	2/32	Negative Control	-
	-	Silymarin	50mg/kg/day
	-	Lactulose	2000mg/kg/day
	-	Silymarin &	50mg/kg/day
		Lactulose	+2000mg/kg/day

Table 2- Effect of thioacetamide, silymarin, lactulose alone or in combination on some serum parameters.

Group	Number of animals	Total bilirubin (mg/dl)	ALB (g/dl)	Total protein (g/dl)	GGT (IU/L)
Control	7	0.227 ± 0.048	3.141 ± 0.434	6.200 ±1.184	3.86 ± 3.185
TAA	7	0.197 ± 0.011	3.498 ± 0.202	7.004 ± 0.629	8.14 ± 5.640 *
SM	7	0.198 ± 0.052	3.088 ± 0.433	6.085 ± 0.915	6.14 ± 5.336
LAC	7	0.285 ± 0.050 * ## \$	3.078 ± 0.531	5.912 ± 1.469	8.14 ± 3.671
SM + LAC	7	0.225 ± 0.023 ¥	2.915 ± 0.760 #	5.590 ± 1.708 #	10.86 ± 7.603 *

Note: Values represent mean ± SD. Values are significantly different (**P* < 0.05 vs. Control), (# *P* < 0.05 vs. TAA), (## *P* < 0.01 vs. TAA), (\$ *P* < 0.05 vs. SM), (¥ *P* < 0.05 vs. LAC).

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Table 3- Effects of treatments on body and liver weights of rats for 8 weeks.

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Groups	Dose (mg/kg/day)	Body weight (g)	Liver weight (g)
Control	-	291.29 ± 13.671	8.045 ± 0.535
TAA	-	287.57 ± 16.582	9.320 ± 1.538
SM	50	284.57 ± 13.661	9.140 ± 1.503
LAC	2000	297.71 ± 15.108	10.195 ± 2.039 *
SM + LAC	50 + 2000	299.00 ± 15.384	10.422 ± 1.808 *
Note: Results were presented as the mean ± SD. (* <i>P</i> < 0.05 vs. Control).			

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Figure captions	568
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Figure1

Figure 2A

Figure 2B

Figure 2C

Figure 3

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Figure 4

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Draft

Figure 5

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Figure 6

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Draft