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Regression of Thioacetamide, Alcohol and Schistosomiasis Induced Liver Fibrosis in Mice by Silymarin

[Farelerde Tiyoasetamid, Alkol ve *Schistosoma Mansoni* Enfeksiyonu ile Oluşturulan Karaciğer Fibrozunun Silimarin ile Gerilemesi]

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ABSTRACT

Objective: This work is aimed to investigate drug-mediated regression of liver fibrosis, generated in mice through intoxication with thioacetamide, ethanol or infection with *Schistosoma mansoni*.

Methods: The study included 120 mice, randomly assigned into 12 groups. In thioacetamide or alcohol-induced liver fibrosis, mice were treated with silymarin during or after intoxication. In schistosomiasis mice, Praziquantel and/or silymarin were used for treatment.

Results: When monitored through biochemical parameters in serum (proteins, bilirubin, Gamma glutamyl transferase and reduced glutathion) and in liver homogenate (malondialdehyde and collagen) the data displayed a similiar regression of fibrosis with the histological parameters (stage and grade). As oxidative stress was the underlying intiator of fibrogenesis; silymarin was able to regress the fibrosis in thioacetamide and alcohol-induced fibrosis. In *S. mansoni* infected mice however, regression was observed in mice treated with a single dose of Praziquantel followed by silymarin. Best improvement was seen in animals treated with the drug(s) after cessation of the fibrotic inducers. Also, the non-invasive liver fibrosis index we developed, confirmed the regression of fibrosis in treated mice versus .

Conclusion: The results indicated an equivalent drug-mediated regression of liver fibrosis initiated by different etiologies, which substantially reduce the possibility of progression to cirrhosis and hepatocellular carcinoma.

Key Words: Liver fibrosis, silymarin, alcohol, Schistosoma mansoni, Thioacetamide.

ÖZET

Amaç: Bu çalışmada farelerde tiyoasetamid ve etanol intoksikasyonu veya *Schistosoma mansoni* enfeksiyonu ile oluşturulan karaciğer fibrozunun ilaç uyarımlı gerileme modelleri incelenmiştir.

Gereç ve yöntemler: Bu çalışmada rastgele olarak 12 gruba ayrılmış 120 fare kullanılmıştır. Tiyoasetamid veya alkol uyarımlı karaciğer fibrozlu farelere zehirlenme sırasında veya sonrasında silymarin uygulanmıştır. Schistosoma'lı farelerde ise tedavi olarak Praziquantel ve/veya silymarin kullanılmıştır.

Bulgular: Bulguların incelenmesi ile serum örnekleri (protein, gama glutamil transferaz ve indirgenmiş glutatyon) ve karaciğer homojenatlarında (malondealdehit ve kollajen) biyokimyasal parametreler açısından gözlenen fibroz gerilemesi histolojik parametreler (evre ve derece) ile tutarlı bulunmuştur. Fibrinogenezi tetikleyici neden oksidatif stres olduğundan silimarin tiyoasetamid ve alkol uyarımlı fibrozu baskılayabilmiştir. *S. Mansoni* ile enfekte farelerde ise tek bir doz praziquantel uygulamsını takiben verilen silmarin ile gerileme gözlenmiştir. En iyi gelişme fibrotik uyarıcıların kesilmesinden sonra hayvanlara ilaç uygulması ile elde edilmiştir. Ayrıca geliştirdiğimiz girişimsel olmayan karaciğer fibroz endeksi de bu gerilemeyi doğrulamıştır.

Sonuçlar: Sonuçlar farklı kökene sahip karaciğer fibrozunun bu ilaç tedavi ise eşit bir biçimde gerilediğini göstermektedir. Bu durum siroz ve hepatosellüler karsinoma gelişimi olasılığını belirgin bir biçimde azaltmaktadır.

Anahtar Kelimeler: Karaciğer fibrozu, silimarin, alkol, Schistosoma mansoni, tiyoasetamid

Introduction

The formation of fibrous tissue is part of the normal beneficial process of wound healing. In pathological conditions however there is an abnormal accumulation of fibrous materials which affect the normal function of the affected tissue. Many common debilitating diseases involve the proliferation of fibrous tissue, the major component of which is collagen (1). Liver fibrosis occurs when the liver is damaged as a result of many risk factors including chronic viral infection with hepatitis viruses (types B or C) (2,3); infection with *Schistosoma mansoni* (4); chemicals (e.g some pharmaceuticals, excessive alcohol intake, pesticides such as thioacetamide (TAA) (5); or due to autoimmune hepatitis (6). Also, fibrosis may be initiated by some metabolic disorders (e.g., lipid, glycogen or metal storage disorders) (7).

Fibrogenesis is a gradual process of increased secretion and decreased degradation of extracellular matrix materials (ECM). The process is initiated by the damage of hepatic cells, which leads to activation and secretion of multiple cellular factors from Kupffer cells. These factors along with the cellular factors secreted by damaged hepatic cells, thrombocytes, endothelial cells of the hepatic sinusoid, and some chemical mediators, are activators of hepatic stellate cells (HSC) which constitute the major source of ECM proteins (8). On activation HSCs differentiate into myofibroblasts and via self- and parasecretion proliferate and synthesize a massive amount of extracellular materials which gradually accumulate and lead to scar formation. Collagens and noncollagenous proteins such as laminin and fibronectin are the main components of the matrix outside the cells; these materials accumulate and stabilize to form fibrous masses (9). This process is controlled by a complex set of proteins, including prolyl 4-hydroxylase, matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) (10).

Oxidative stress, steatosis, cytokines, growth factors, enzymes and enzyme inhibitors play a pivotal role in fibrogenesis (11). Consequently, complete recovery from liver fibrosis would involve the breakdown of multiple ECM components with degradation of the predominant component being particularly important for recovery of normal liver histology (12). Effective treatment of the underlying insult is available, where remodeling of the scar tissue and the return towards normal architecture have been documented even in advanced fibrosis. This was reported in autoimmune disease (13) and in patients with hepatitis B and C (14) after successful interferon therapy. In addition, another traditional medicine therapy (using herbal extracts) gave positive results in hepatitis B patients (15, 16).

Since lipid peroxidation and oxidative stress are the common factor in theetiology of diffrent liver fibrosis, there is a possible of treatment of fibrosis with antioxidants. The flavonoid silymarin has hepatoprotective properties. Its mechanisms of action are still poorly understood. However, the data in the literature indicate that silymarin acts in four different ways: (i) as antioxidant, scavenger and regulator of the intracellular content of glutathione; (15) (ii) as cell membrane stabiliser and permeability regulator that prevent hepatotoxic agents from entering hepatocytes; (iii) as promoter of ribosomal RNA synthesis, stimulating liver regeneration and (iv) as inhibitor of the transformation of stellate hepatocytes into myofibroblasts, the process responsible for the deposition of collagen fibers leading to cirrhosis (16).

Thus this study aimed to investigate the possibility of fibrosis regression in etiologically different liver fibrotic mice models generated by intoxication with thioacetamide (TAA), alcohol or infection with *Schistosoma mansoni*. In parallel to the invasive histological analysis, we applied a non-invasive fibrosis index to assess the degree of fibrosis.

Materials and Methods

Animal grouping:

The study employed 120 albino mice (MC1 strain), weighing 17.78-26.62 g, kept in breeding cages and received a similar basic care with standard diet. Mice were grouped (according to the liver fibrosis inducer and the therapy they received) into 12 groups (10 mice each). Group I included normal control mice. Group II mice were injected with TAA. Group III was simultaneously treated with both TAA and silymarin, whereas group IV mice were injected with TAA, for a month, then silymarin for a similar period. Group V mice were infected with *S. mansoni* ; in group VI after being infected with *S. mansoni*, the mice were treated with Praziquantel (PZQ). Group VII was treated with silymarin for a month, starting after thge date of infection with *S. mansoni.* After being infected with *S. mansoni*, groups VIII and IX mice were treated only with silymarin or with PZQ and silymarin, respectively. Group X mice was intoxicated with ethanol, whereas groups XI and XII mice were treated with silymarin during or after ethanol treatment, respectively. Figure 1 shows the time course of fibrogenesis and treatments in different groups.

Sampling

At the end of treatment period, animals were sacrificed, where the blood and liver tissues were collected. Serum was recovered for biochemical investigations and liver homogenate was prepared and used in determination of hepatic parameters. Also, a portion of liver was kept in 10 % formalin and used for histopathological analysis.

Induction of fibrosis and treatment

TAA-induced liver fibrosis was generated in mice by intrapretoneal injection of 200 mg/kg TAA (Sigma-Aldrich Chemical Co., USA) twice a week for a month (17).

S. mansoni-induced liver fibrosis was generated in mice by immersion of tails into a suspension of 50 cercaria for 30 minutes. Cercaria develops into larva, oocyte then progresses into adult worm in about 42 days (18). To develop ethanol-induced liver fibrosis, ethanol (10 %) was given to the mice in drinking water for 35 days (19). *Silymarin* (Sedico Co., ARE), dissolved in water, was orally taken with concentration 140 mg/dl (13). As a reference antischistosomiasis drug, mice were treated with a single dose (600 mg/kg of mice body weight) of PZQ (Biltricide, Epico Co., ARE) given by gavage.

Biochemical investigations

Serum bilirubin was estimated according to Jendrassik L and Grof (20) using the commercial kits (Diamond Co.); Gamma glutamyl transferase (E.C.2.3.2.2.) activity was estimated according to Szasz and others (21) using the commercially available kits (Spectrum, Co.) following the manufacturer instruction and total proteins was estimated according to Bradford, (22) using the commercially available kits. Reduced glutathione (GSH) in serum was estimated by the method of Ellman (23), based on the oxidation of GSH by 5,5'-dithiobis-2-nitrobenzoic acid, [DTNB] to produce a yellow colored ion, which absorbs light at 412 nm. Liver homogenate (10 %) was used for to determine hepatic hydroxyproline (HP) and collagen. HP was hydrolyzed with HCl, oxidized into pyrole and coupled with *p*-dimethyl-aminobenzaldehyde (Sigma-Aldrich Chemical Co., USA) forming a red color measured at 558 nm (24, 25). The collagen content was calculated by multiplying the HP concentration by 7.46, where this imino acid represents 13.4 % of collagen. Total malondialdehyde (MDA) (free and protein-pound Schiff base conjugates) was estimated according to Okhawa H and Ohishi N (26). The method depends on the reaction of MDA with thiobarbituric acid (TBA) (Sigma-Aldrich Chemical Co., USA).

Histological and non-invasive scoring of liver fibrosis.

The standard protocol of tissue staining by hematoxyline and eosin (27) was applied and slides were examined and reviewed by a pathologist, where both stage and grade were determined. Also, the degree of fibrosis was scored according to the logarithmic formula we derived (unpublished work) from a combination of four serum markers (Proteins, Bilirubin, γ–GT and GSH) of both normal and investigated mice. The score ranges from "0" for healthy liver to "2" for invasive fibrosis.

Statistical analysis

Results were presented as a mean (± standard deviation) and the difference between groups was tested by student t-test or one-way ANOVA test. P values less than 0.05 were considered significant. Statistical analysis was performed using Graphpad instat software package (Graphpad, San Diego, CA, USA).

Results:

Improvement of the biochemical parameters

A selected biochemical panel was used to monitor the effect of fibrotic inducers in both serum and liver. The panel includes serum γ-GT, bilirubin, total protein, GSH, and as hepatic parameters include MDA and collagen. As table 1 shows, the serum level of bilirubin (TBili) dramatically increased in mice fibrogenated with TAA, *S. mansoni* and alcohol (0.19±0.02, 0.127±0.02 and 0.162±0.02 mg/dl, respectively) as compared to the baseline of normal control mice (0.015±0.01 mg/dl). When mice was treated with silymarin and/or PZQ, a variable pattern of reduction in TBili was observed. The decrease ranged between 81 % and 13 % of the level of untreated mice. Best improvement was achieved in mice treated with silymarin after cessation of TAA or ethanol intoxication (groups IV and XII) and in mice dually treated with silymarin and PZQ after *S. mansoni* infection, where TBili was 13 %, 17 % and 13.7 % of the corresponding untreated mice. Less improvement was noticed in animals protected with the drug(s) during the fibrogenesis process.

In post-fibrosis treated mice (groups IV, IX and XII), γ-GT decreased to 10.42 ± 0.82, 9.73 ± 0.63 and 10.19 ± 0.97 U/L, similar to the normal mice (9.27 ± 0.83 U/L). Also, the depletion of serum proteins seen in mice with fibrosis (3.83 ± 0.1, 4.74 ± 0.22 and 4.31 ± 0.2 g/dl) increased to levels comparable to that of healthy animals. Revealing the development of oxidative stress in mice with liver fibrosis (groups II, V and X), serum GSH were restored in mice treated with silymarin and/or PZQ after cessation of the fibrotic inducers TAA, *S. mansoni* and alcohol, respectively (Table 1). In parallel, the increased hepatic MDA seen in fibrogenated animals (indicating the oxidative stress in liver), (Table 2) was improved with silymarin, particularly in TAA and alcohol-induced fibrosis. In *S. mansoni* infected mice, neither silymarin nor PZQ (alone) had a significant effect on hepatic MDA, where they had minimal effect (11 % and 5 %, respectively) on MDA decrease. The improvement in both liver function and oxidative stress condition, significantly limited the over production of hepatic collagen, which decreased to 122.14±10.7, 118.88±9.76 and 119.21±13.55 µg/g (more or less similar to that of healthy animals, 110.80±9.2 µg/g). The overall picture indicates the efficacy of silymarin and PZQ to ameliorate the liver condition regardless of the etiology of fibrosis.

Regression of fibrosis stage and inflammation in liver

In general the histological analysis of liver fibrosis in treated mice, revealed the regression of both the stage

Table 1: Serum levels of liver function and oxidative stress markers.

Fibrotic inducer	Group No. (treatment)	Bili (mg/dl)	GGT (U/L)	TP (g/dl)	GSH (mM)
	I (Normal) w *(n=10)*	0.015 ± 0.006	9.27 ± 0.83	6.10 ± 0.10	1.42 ± 0.04
TAA	II (TAA) *(n=10)*	0.19 ± 0.017a	28.49 ± 1.32a	3.83 ± 0.17a	0.82 ± 0.02a
	III (TAA sily) *(n=10)*	0.037 ± 0.006ab	13.9 ± 0.82ab	5.67 ± 0.22ab	1.34 ± 0.01ab
	IV (TAA then Sily) *(n=10)*	0.024 ± 0.009b	10.42 ± 0.82b	5.98 ± 0.13b	1.38 ± 0.03b
S. mansoni	V (S. mansoni infection). *(n=10)*	0.127 ± 0.019a	22.93 ± 0.52a	4.74 ± 0.22a	1.23 ± 0.02a
	VI (PZQ after infection) *(n=10)*	0.103 ± 0.019a	22.00 ± 0.63a	4.80 ± 0.23a	1.24 ± 0.03a
	VII (Sily. during infection) *(n=10)*	0.054 ± 0.008ab	16.91 ± 1.04ab	5.25 ± 0.18ab	1.33 ± 0.03ab
	VIII (Sily. after infection) *(n=10)*	0.065 ± 0.008ab	18.30 ± 0.97ab	5.07 ± 0.11ab	1.31 ± 0.01ab
	IX (Silymarin after PZQ) *(n=10)*	0.017 ± 0.006b	9.73 ± 0.63b	5.95 ± 0.15b	1.41 ± 0.02b
Ethanol	X (Ethanol) *(n=10)*	0.162 ± 0.017a	26.17 ± 1.32a	4.31 ± 0.20a	0.99 ± 0.03a
	XI (Sily during ethanol intake) *(n=10)*	0.028 ± 0.006ab	11.12 ± 1.04ab	5.85 ± 0.23ab	1.34 ± 0.01ab
	XII (Sily after ethanol intake) *(n=10)*	0.022 ± 0.008b	10.19 ± 0.97b	5.95 ± 0.35b	1.40 ± 0.02b

Bil: bilirubin, γGT: gamma glutamyltranseferase, TP: Total protein and GSH: reduced glutathione, PZQ : Prazaquantel ; Sili.: silymarin
Small letters (a) and (b) indicate a significant change of the corresponding group compared to normal and untreated groups, respectively.
Mean values of groups were compared by ANOVA test.

and grade. Livers of positive control animals for TAA mice, *S. mansoni*, alcoholism were in stages 3, 2 and 3, and grades 3, 4 and 3 respectively. In TAA-induced fibrosis (gp II) for example, histology revealed porto-portal bridging in portal tracts, portal fibrosis and piecemeal necrosis, fatty changes in hepatocytes, portal inflammation and congested central vein. When silymarin was prophylactically taken (group III), the condition was moderately improved showing inflammatory reactions in portal tracts, fatty changes in hepatocytes, no bridging in portal tracts and the nodularity in hepatocytes surface was less. When the drug was taken after cessation of TAA (group IV), livers had enlarged hepatocytes, less inflammatory reactions in portal tracts and less congestion in central vein. The same regression pattern was observed in the alcoholism groups. Slides from *S. mansoni* infected mice (group V) demonstrated the existence of the ova of *S. mansoni* and it was surrounded by severe inflammatory reaction (grade 4) and congested central vein (Fig. 3). PZQ (in group VI) slightly relieved the inflammatory reaction (grade 2), and more improvements were obtained with the dual treatment of PZQ and silymarin, whereas normal histological pattern with normal hepatocytes and few inflammatory reactions in portal tract without ova or adult worm was seen in mice treated with both drugs after cessation of *S. mansoni* (group XII).

Biochemical versus histological scoring

The histological analysis implemented in different groups was paralleled by using a non-invasive index developed by us to score the degree of fibrosis. This index is based on the combination of four serum markers (protein, Bil, γ-GT and GSH) in a logarithmic formula ranging from "0" to "2". Values under 0.24 corresponds to normal liver histology observed in normal control mice. Mild fibrosis was indicated by values between 0.25 and 1.1 corresponding to the histological stage "1" (as seen in groups III, IV, VII, VIII, IX, XI and XII). Higher values (1.12 up to 1.8) correspond to moderate fibrosis (S2) (groups V and VI), whereas the highest values correspond to invasive fibrosis (stage 3) as seen in TAA, ethanol intoxicated or S. mansoni infected groups without treatment. The index highly correlated with the corresponding histological stage (data not shown). In general monitoring the effect of drug-mediated regres-

Table 2: Concentrations of liver and oxidative stress markers in liver tissue.

Fibrotic inducer	Group No. (treatment)	HP	Collagen	TP	MDA
	I (Normal) (*n=10*)	14.84 ± 1.24	110.80 ± 9.22	24.48 ± 1.21	0.83 ± 0.02
TAA	II (TAA) (*n=10*)	42.65 ± 3.65a	318.19 ± 27.2a	11.76 ± 1.37a	2.15 ± 0.12a
	III (TAA sily) (*n=10*)	17.33 ± 1.97ab	129.24 ± 14.66ab	21.90 ± 1.56 ab	0.99 ± 0.07ab
	IV (TAA then Sily) (*n=10*)	16.14 ± 1.43b	122.14 ± 10.70b	23.60 ± 1.33 b	0.87 ± 0.04 b
S. mansoni	V (S. mansoni infection). (n=10)	32.40 ± 2.35a	241.83 ± 17.50a	16.73 ± 2.57a	1.39 ± 0.14a
	VI (PZQ after infection). (*n=10*)	31.72 ± 2.68a	236.62 ± 20.02a	17.04 ± 1.68a	1.32 ± 0.05a
	VII (Sily. during infection). (*n=10*)	19.28 ± 1.35ab	143.84 ± 10.08ab	19.65 ± 1.65ab	1.09 ± 0.07a
	VIII (Sily. after infection) (*n=10*)	21.92 ± 1.54ab	163.51 ± 11.47ab	18.43 ± 1.39ab	1.14 ± 0.05a
	IX (Silymarin after PZQ) (*n=10*)	15.94 ± 1.31b	118.88 ± 9.76b	24.20 ± 0.98b	0.85 ± 0.01b
Ethanol	X (Ethanol) (*n=10*)	37.96 ± 3.20a	283.19 ± 23.88a	13.65 ± 1.91a	1.74 ± 0.06a
	XI (Sily during ethanol) (*n=10*)	17.70 ± 1.58ab	132.04 ± 11.79ab	22.21 ± 1.56ab	0.95 ± 0.02ab
	XII (Sily after ethanol intake). (n=10)	15.98 ± 1.82b	119.21 ± 13.55b	23.65 ± 1.19b	0.87 ± 0.03b

HP: hydroxyprolin, TP: total protein, MDA: malondialdehyde, PZQ : Prazaquantel; Sili.: silymarin.
Small letters (a) and (b) indicate a significant change of the corresponding group compared to normal and untreated groups, respectively.
Mean values of groups were compared by ANOVA test.

sion of fibrosis was more or less similar in mice exposed to different fibrotic factors.

Discussion

Due to the unavailability of animal model for HBV or HCV infection, viral hepatitis-induced liver fibrosis was not included. The study was restricted to fibrosis experimentally induced with three different causes including TAA, chronic alcohol intake and infection with *S. mansoni*. In literature there are repeated reports on liver fibrosis in HBV and/or HCV infected patients, however few studies have investigated the regression of fibrosis induced by alcoholism, *S. mansoni* infection or exposure to hepatotoxic agent.

Among other hepatotoxins, TAA is the most potent because of its rapid elimination and cumulative injury (28). In addition to the oxidative stress it generates, TAA decreases the level of some endogenous antioxidants including α-tocopherol (29). Consequently, it is anticipated that supplementation of mice with an antioxidant (gps III and IV), could compensate the depletion of the vitamin and challenge the oxidant effect. The oxidative effect of TAA was observed in both serum (GSH) and liver (hepatic MDA). Also, the inflammation in liver tissues was noticable as an increase of the histological grade. The deteriorative effects of TAA on both liver function markers and the oxidative stress it generates was pronounced; 12-fold and in bilirubin and 3-fold increase in γ-GT was noted whereas significant decrease in both total protein and GSH levels was observed. Although MDA, an end product of lipid peroxidation, is usually broken down by endogenous aldehyde dehydrogenases in the liver (29), its production however was accelerated (more than 2.5 fold increase) by TAA so much that it may escape the detoxification process. Such lipid peroxidation-mediated oxidative stress was suggested to be generated by TAA-S-oxide, which is derived from the biotransformation of TAA by the liver microsomal FAD-containing monooxygense (FMO) and cytochrome P450 systems (5, 30). This condition explains the activation of HCS which overproduced hepatic collagen (2.8 fold-increase in TAA injected mice compared to both normal and silymarin treated animals). Beside biochemical alterations, the histological analysis has confirmed the development invasive fibrosis (stage 3 and grade 3) in mice treated with TAA (Figures 2 and 3).

Schistosomiasis, on the other hand caused periportal fibrosis due to deposition of eggs in the small portal venules (31). Hepatic involvement is considered the most

serious complication because the parasite lives in the portal circulation and some ova are occasionally swept back to liver inducing granulomatous reactions (32). In the liver the initial pathologic response is an immunologic reaction to antigens secreted by the organism inside the eggs (33). In addition, the host reaction to the eggs lead to an extensive damage of the hepatoportal vascular system and subsequent fibrous scar formation (18). Compared to the corresponding level on normal mice, *S. mansoni* infection led to 2.5 and 8-fold increase in serum γ-GT and bilirubin and twice-fold decrease in total proteins and GSH levels. Alcoholism can also cause liver inflammation because alcohol and its metabolite are toxic to liver cells. In agreement with previous reports (34, 35), ethanol led to a drastic increase in hepatic MDA, decrease of GSH, increase of γ-GT and bilirubin. Mechanistically, alcohol intake has a complex pathogenesis, in which acetaldehyde (AcCHO), the major metabolite, plays a central role. Alcohol is mainly metabolized in the liver by two oxidative pathways. The first is the oxidation of ethanol to acetaldehyde by the cytoplasmic alcohol dehydrogenase (ADH), and acetaldehyde is then oxidized to acetic acid by the mitochondrial acetaldehyde dehydrogenase (ALDH). The second pathway involves the microsomal ethanol-oxidizing system (MEOS), in which the oxidation to acetaldehyde and acetic acid also leads to generation of reactive oxygen species (ROS). ROS enhances HSC activation and stimulates fibrogenesis (36). The overall picture shows that the three factors we used (TAA, *S. mansoni* and ethanol) effectively induced liver fibrotic models which has been used to address whether liver fibrosis induced by different fibrotic factors is reversible.

From the biochemical point of view, fibrosis is considered the net result of the imbalance between the collagen synthesis and degradation. When synthesis is very active and the decomposition is suppressed, fibrosis will progress. Vice versa, fibrosis could be reversed if the inflammation is controlled. The treatment strategy conducted in this work had two formats: the first was to antagonize the fibrotic factor with drug(s). This was investigated in mice simultaneously treated with the fibrotic factor and silymarin and/or PZQ. The second format was to pause the effect of the liver insulting agent and to start treatment where animals were treated with drug(s) after the fibrosis was established.

Because the oxidative stress is the underlining mechanism in all models, silymarin was chosen to reverse the condition. Also, treatment by PZQ (37) was used in schistosomiasis mice, where specific eradication of the parasite was necessary to enhance the effect of silymarin. In all treated groups the regression of fibrosis was monitored both biochemically and histologicaly. As the data showed bilirubin was decreased by 81 % and 87 % when silymarin was taken during and after TAA, respectively, as compared to 78 % and 83 % in mice intoxicated with ethanol. In *S. mansoni* infected mice, bilirubin was improved with a lower percent (49 %) when animals were treated only with silymarin and it was increased by 87 % in case of treatment with both silymarin and PZQ. The hepatoprotective activity of silymarin against ethanol-induced damage (seen in gps III and IV) was observed through the significant improvement in both serum and hepatic markers. In consistance with previous reports (38)**,** effective improvement of the bilirubin was observed when drug(s) were taken after cessation of the insulting factors. PZQ, however alone slightly improved the bilirubin level by 19 % (in gp VI). Also, silymarin alone improved it by 48 % or 49 % (gp IIV and VIII) when taken alone during or after TAA, respectively. The abnormally high γ-GT level was completely restored to normal, particularly in mice treated with silymarin after TAA and ethanol intoxicated mice. The γ-GT levels in *S. mansoni* animals was similar to bilirubin where treatment of infected animals with PZQ then silymarin was required to normalize the level of γ-GT. The compensated levels of GSH in serum and MDA in liver confirmed the silymarin-mediated removal of the oxidant effects. More importantly the process of fibrogenesis, which is frequently explained by the over production of ECM proteins, was ameliorated as indicated by the normal collagen content in livers of treated mice (gps IV, IX and XII).

The biochemical changes seen in treated mice were paralleled with improved phenotypical changes represented by the regression of both the histological stages and grades (Fig. 2). The highest fibrotic stage was observed in untreated mice. Different treatments led to regression of the fibrotic stages to moderate or mild fibrosis The pre-cirrhotic stage (S3) of TAA and alcoholism induced-liver fibrosis, was improved to mild, or no fibrosis stage (S1or S0", respectively) in silymarin treated mice. Also, complete recovery of liver was observed when PZQ and silymarin were used to treat mice with liver fibrosis due to infection with *S. mansoni*. This may indicate a different responsiveness between animal models and human. In addition to the histological analysis, we used a non-invasive index to monitor the degree of liver fibrogenesis. The data obtained by both traditional analysis and serum-based index (B. score) were highly correlated in different groups, where the highest scores (>1.8) matched the histological stages S3, in groups II, V and X which included fibrogenated mice. A marked regression of fibrosis index was obtained in treated mice (Fig. 2).

In conclusion, the study provides a model of drug-mediated resolution of liver fibrosis mimicing the trials of drug-mediated treatment of fibrosis in HCV patients. As the oxidative stress was the underlying scenario of scar formation, treatment with a potent antioxidant (such as silymarin) in addition to specific anti-parasitic treatment (in case of schistosomiasis), effectively regresses the biochemical and phenotypical alterations in pre-cirrhotic livers.

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