Nigella sativa seed reduced galectin-3 level and liver fibrosis in thioacetamide-induced liver injury in rats

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Background/aim
Fibrosis represents the final common pathway of chronic tissue injury. Galectin-3 (Gal-3) is an important regulator of fibrosis that links chronic inflammation to fibrogenesis. We investigated the effect of *Nigella sativa* seed (black seed), a common hepatoprotective natural remedy, on Gal-3 level and progression of liver fibrosis in thioacetamide (TA)-induced liver injury in rats.
Materials and methods
Forty male Wistar rats were used in this study and were divided into four groups of 10 rats each. Group I served as control, groups from II to IV were intoxicated by TA (200 mg/kg body weight); meanwhile group III was treated with silymarin (50 mg/kg body weight) and group IV was treated with black seed (50 mg/kg body weight). Gal-3, transforming growth factor β1 (TGF-β1), some antioxidant and oxidative stress biomarkers were determined in the liver tissue homogenate. Moreover, serum liver function parameters, total cholesterol, triacylglycerols, and plasma glucose were determined. Quantitative measurement of fibrotic areas was achieved using computerized image analysis system.
Results
TA administration caused significant elevations in the levels of liver Gal-3, TGF-β1, malondialdehyde, nitric oxide and serum alanine transaminase and aspartate transaminase activities, total bilirubin, total cholesterol, triglycerides, and plasma glucose levels. Meanwhile, significant decreases were recorded in the level of liver total antioxidant capacity, catalase activity, and serum levels of total protein and albumin. Histopathological observation showed severe damage in the liver and presence of fibrotic areas. Treatment with silymarin and black seed resulted in decreasing levels of liver Gal-3 and TGF-β1 and marked improvement in liver functions, as well as reducing the fibrotic areas in the liver. Gal-3 exhibited positive correlation with TGF-β1, malondialdehyde, nitric oxide, alanine transaminase and aspartate transaminase, while it negatively correlated with total antioxidant capacity and catalase.
Conclusion
Black seed reduced liver Gal-3 level and ameliorated fibrogenesis in liver due to TA administration.

Keywords:
black seed, galectin-3, liver fibrosis, rats, thioacetamide

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Introduction

Many people around the world have been afflicted by some type of liver lesions including fatty liver, nonalcoholic steatohepatitis, hepatitis with its types (A, B, and C), cirrhosis and hepatocellular carcinoma [1]. Fibrosis, and in certain, cirrhosis represent the final endpoint of chronic liver tissue injury, in which chronic inflammation leads to the formation of scar tissue, loss of tissue architecture, and organ failure [2]. Liver fibrosis is one of the leading causes of morbidity and mortality worldwide [3].

Until now there is no effective and reliable drug for resolving fibrosis. However, there are many trials depending on approaches using several agents that block fibrogenic activation and extracellular matrix (ECM) production by myofibroblasts. This works well in culture and in some rodent models of liver fibrosis but carry a high risk of unwanted side effects in patients due to a lack of specificity for myofibroblasts [4,5].

Galectins are a category of animal lectins that can bind β-galactosides through their carbohydrate recognition domain, and each galectin prefers binding to an

individual carbohydrate. They can be found inside cells in the cytoplasm and nucleus or in the ECM, and can also be found on the cell surface [6].

Among all the galectins, galectin-3 (Gal-3) was found to have important role in many biological functions such as, cell adhesion, cell signaling, cell proliferation, apoptosis and angiogenesis, immune reactions and fibrogenesis [7].

It is believed that the soluble part of Gal-3 is responsible for myofibroblast activation, leading to collagen secreting in the ECM and fibrogenesis. Also Gal-3 expression has been accompanied with the development of fibrosis in a variety of organs, such as the liver, kidney, gut, and heart [7,8]. Gal-3 has a role in thioacetamide (TA) acute liver injury through the production of monocyte chemoattractant protein-1 and transforming growth factor-β1 (TGF-β1) [9]. TGF-β1 is a cytokine that enhances fibrosis in many organs. It is mainly produced by hepatic stellate cells and induces the production of ECM components [10].

Nigella sativa seed (black seed) is a widely used natural food and medicinal plant throughout the world. It has been used in traditional medicine in Middle and Far East since ancient times [11]. Black seed contains many active components such as thymoquinone (30–48%), p-cymene (7–15%), and carvacrol (6–12%). It is reported to have a wide spectrum of therapeutic properties such as antihypertensive, antidiabetic, immunomodulatory, anticancer, renalprotective and hepatoprotective, anti-inflammatory, and antioxidant properties [12–15]. Many studies have shown the protective role of *N. sativa* against heavy metals, such as lead and cadmium, toxicity in liver tissues. Other studies reported the protective effect of *N. sativa* against hepatic lipid peroxidation introduced by toxic compounds, such as carbon tetrachloride [16].

TA is a hepatotoxicant that causes centrilobular necrosis in the liver. TA is widely used as a model compound for induction of acute and chronic liver injury and fibrosis, which closely resembles human micronodular cirrhosis [17,18].

Silymarin is among the drugs that are used in the treatment of hepatic dysfunction. Silymarin is a mixture of flavolignans and flavonoids with antioxidant properties, extracted from the plant *Silybum marianum* [19]. It is used as a hepatoprotective agent against hepatic injury caused by many toxic substances such as CCl_4, aflatoxin B1, and galactosamine [20].

This study was carried out to evaluate the effect of black seed on Gal-3 level in rats suffering from TA-induced hepatic injury. The study also investigated the correlation between Gal-3 level and other biochemical markers for liver injury. Additionally, this study compared the results with the standard drug, silymarin.

Materials and methods

Black seed and silymarin used

Black seed was purchased from the Ministry of Agriculture Selling Port, Giza, Egypt. The black seed was washed, dried, ground, and then suspended in water before use. Silymarin was obtained from the pharmacy as 10 sachets produced by SEDICO Pharmaceutical Co., 6 October City, Egypt. Each sachet contains 140 mg silymarin (calculated as silybin). It was freshly prepared and administered by dissolving the content of each sachet in water (50 ml).

Experimental animals and diet

A total of 40 male Wistar rats (90–130 g) were obtained from the National Research Centre, Animal House. The animals were maintained under standardized environmental conditions at 12 h light/dark cycle and at a constant temperature of 25±1°C. Animals were fed on basal diet [21,22] and water was supplied *ad libitum*. Rats were acclimated to laboratory conditions 1 week prior to the beginning of the experiment.

Experimental design

The animals were segregated into four groups, 10 rats per each, as follows. Group I (normal controls): rats received saline intraperitoneally (0.01 mg/kg body weight); group II (TA intoxicated group): rats injected intraperitoneally by 200 mg/kg body weight of TA two times per week for 7 weeks [23]; group III (silymarin and TA supplemented group): rats were treated with TA as in group II and received orally 50 mg/kg body weight of silymarin daily for 7 weeks; group IV (*N. sativa* and TA supplemented group): rats were treated with TA as in group II and received orally 50 mg/kg body weight of *N. sativa* daily for 7 weeks [24].

Collection of blood samples

At the end of the experiment, blood samples were collected after 16 h fasting using the orbital sinus technique of Sanford [25]. Blood samples were left to clot in clean dry test tubes, and then centrifuged at 3000 rpm for 10 min. The clear supernatant serum was then separated and frozen at –20°C for the biochemical

analysis. A small portion of blood was withdrawn into sodium fluoride coated tubes for determination of glucose level. After blood collection, the rats were killed by decapitation and the whole liver of each animal was rapidly dissected, thoroughly washed with isotonic saline and plotted. A small part of each liver was cut and weighed for preparing liver tissue homogenate; the rest of each liver was fixed in formaldehyde buffer (10%) for histological investigation.

Biochemical analyses methods

Liver Gal-3 and TGF-β1 level were determined by enzyme-linked immunosorbent assay technique, using the kit purchased from Sun Red Biotechnology (Shanghai, China), according to the method described by the manufacturer. Liver total antioxidant capacity (TAC), catalase, malondialdehyde (MDA), and nitric oxide (NO) were estimated using commercial kits purchased from Biodiagniostic, Egypt, based on the methods described by Koracevic *et al.* [26], Aebi [27], Satoh [28], and Montgomery and Dymock [29], respectively.

Serum alanine transaminase (ALT) and aspartate transaminase (AST) were estimated using kits of Vitro Scient (Hannover, Germany), based on the method described by Bergmeyer *et al.* [30] and Henry *et al.* [31], respectively. Serum total bilirubin was determined using the kit of Biodiagnostic (Dokki, Giza, Egypt), based on the method described by Walter and Gerade [32]. Serum total protein, albumin, and plasma glucose were estimated using kits purchased from Spectrum (Hannover, Germany), based on the methods described by Cannon *et al.* [33], Doumas *et al.* [34], and Howanitz and Howanitz [35], respectively. Serum total cholesterol and triacylglycerols were estimated using kits of Vitro Scient, based on the methods described by Allain *et al.* [36] and Fossati and Prencipe [37], respectively.

Histopathological examinations and image analysis

The liver of rats of different groups were removed and fixed in 10% formal saline; 5 μm thick paraffin sections were stained with haematoxylin and eosin [38] and examined by light microscope. Formalin-fixed specimens were embedded in paraffin, sliced with a thickness of 4 μm and mounted on silanized glass slides. Van Gieson's staining, described by Bancroft and Cook [39], was performed for quantitative measurement of fibrotic areas by using computerized image analysis (Leica Qwin 500) in Image Analyzer Unit, Pathology Department, National Research Centre. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Ten nonoverlapping fields were chosen in each specimen and the mean values were obtained.

Statistical analysis

The data obtained in the present work are represented as average mean±SE. Statistical analysis was evaluated using the Student *t*-test. *P* values of less than 0.05 were considered statistically significant [40].

Results

Biochemical analyses results

The results obtained in Table 1 show liver Gal-3, TGF-β1, TAC, MDA, NO levels, and catalase activity. Liver Gal-3 and TGF-β1 levels exhibited significant increase ($P<0.05$) in groups II–IV when compared with normal controls (group I) and significantly decreased ($P<0.05$) in groups III and IV when compared with group II. Meanwhile, both the liver TAC level and catalase activity decreased significantly ($P<0.05$) in groups II and IV when compared with group I, while both of them increased significantly ($P<0.05$) in groups III and IV when compared with group II. Concerning liver

Table 1 Liver levels of galectin-3, transforming growth factor-β1, antioxidant, and oxidative stress parameters in the experimental groups

Parameters	Groups			
	Control (group I)	Thioacetamide (group II)	Thioacetamide+silymarin (group III)	Thioacetamide+black seed (group IV)
Galectin-3 (ng/mg tissue)	2.88±0.12	4.49±0.16 (55.90)[a]	3.56±0.25 (23.61)[a,b]	3.80±0.28 (31.94)[a,b]
TGF-β1 (ng/mg tissue)	41.60±2.06	73.11±6.59 (75.75)[a]	55.34±1.72 (33.03)[a,b]	56.49±3.20 (35.79)[a,b]
TAC (mmol/mg tissue)	11.09±0.17	4.61±0.26 (–58.43)[a]	10.65±0.17 (–3.97)[b]	7.82±0.18 (–29.49)[a,b]
Catalase (Ul/g tissue)	3755±156	2056±240 (–45.25)[a]	3485±133 (–7.19)[b]	2837±156 (–24.45)[a,b]
MDA (nmol/g tissue)	1193±108	5503±152 (361.27)[a]	1688±57 (41.49)[a,b]	2993±96 (150.88)[a,b]
NO (μmol/g tissue)	1194±32	3012±45 (152.26)[a]	1440±53 (20.60)[a,b]	2280±76 (90.95)[a,b]

All data are represented as mean±SE; MDA, malondialdehyde; NO, nitric oxide; TAC, total antioxidant capacity; TGF-β1, transforming growth factor β1; [a]Significant difference versus control group at $P<0.05$; [b]Significant difference versus thioacetamide intoxicated group at $P<0.05$. Numbers between brackets represent percent change from control group.

MDA and NO levels, there was a significant increase ($P<0.05$) of their levels in groups II–IV when compared with group I; meanwhile, both of them decreased significantly ($P<0.05$) in groups III and IV when compared with group II.

Table 2 exhibits the results of serum liver function parameters, total cholesterol, triacylglycerols, and plasma glucose. It was found that each of serum ALT and AST activities and total bilirubin level increased significantly ($P<0.05$) in groups II–IV when compared with group I, while they decreased significantly ($P<0.05$) in groups III and IV when compared with group II. In comparison with group I, serum total protein and albumin levels of group II decreased significantly ($P<0.05$); however, both of them increased significantly ($P<0.05$) in group IV. Meanwhile both levels increased significantly ($P<0.05$) in comparison with group II. Regarding serum total cholesterol, triacylglycerols, and plasma glucose, their levels increased significantly ($P<0.05$) in group II when compared with group I. Moreover, the levels of the three parameters in groups III and IV decreased significantly ($P<0.05$) when compared with group II.

Histopathological examinations and image analysis

Liver sections from normal control rats exhibit normal structure of hepatic lobules (Fig. 1a). Examination of the liver sections from TA intoxicated rats showed nodular appearance of the liver lobule, bridging necrosis accompanied with mononuclear inflammatory cells, signs of vacuolar degeneration of hepatocytes with balloon cells, and focal proliferation of biliary ductules within the periportal tracts (Fig. 1b).

Liver sections of rats given silymarin and TA showed acute inflammatory infiltrate within the periportal tracts entering focally the liver lobule and vacuolar degeneration of single hepatocytes. Fibrotic septa were seen (Fig. 1c). In some rats from the same group, mild inflammation but no fibrotic septa were shown. Hepatocytes appeared more or less like normal ones with small areas of hydropic degeneration (Fig. 1d).

The histopathological study of liver sections from *N. sativa* and TA supplemented group showed mild inflammation in the portal and the periportal tract without fibrotic septa. Hepatocytes appear more or less like normal ones (Fig. 1e and f).

Liver sections stained with Van Gieson's stain from normal control rats show the normal hepatocytes architecture and mild fibrotic area within the periportal vein (Fig. 2a).

Examination of the liver sections stained with Van Gieson's stain from TA intoxicated rats indicated intense fibrosis that divide the liver lobules into rounded contours or visible nodules (Fig. 2b). Image analysis showed significant increases in the areas of fibrosis in the group of rats treated with TA alone as compared with control. The mean of fibrotic areas found in the examined rat liver sections was 1247±335 μm^2 (Table 3).

Using Van Gieson's stain, liver sections of TA and silymarin group showed moderate thin septa (Fig. 2c). In some sections, red positive fibrotic areas indicated mild fibrosis within the periportal tract entering the liver lobules (Fig. 2d). In addition, the fibrotic areas calculated from the examined liver sections showed a significant decrease ($P<0.05$) in comparison with the TA group (Table 3).

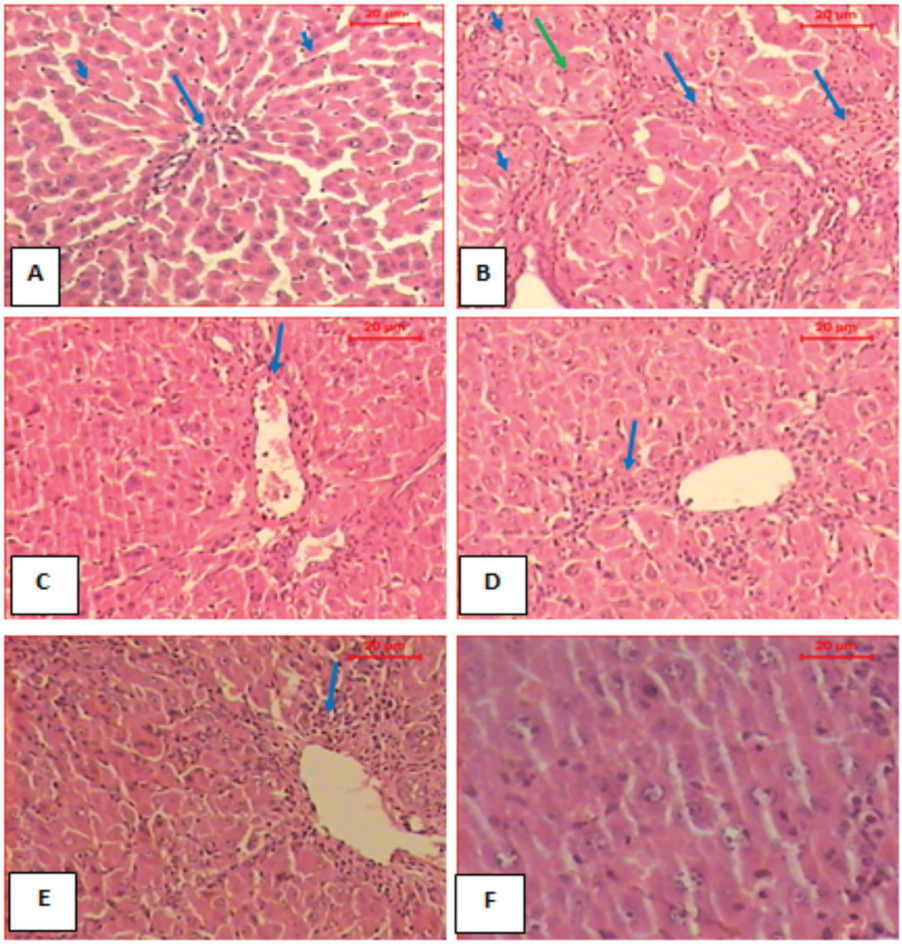
On the other hand, the liver sections stained by Van Gieson's stain in the *N. sativa* and TA group

Table 2 Serum levels of liver function parameters, total cholesterol, triacylglycerols, and plasma glucose in the experimental groups

Parameters	Groups			
	Control (group I)	Thioacetamide (group II)	Thioacetamide+silymarin (group III)	Thioacetamide+black seed (group IV)
ALT (UI/l)	47.17±2.43	167.48±6.42 (255.06)[a]	68.97±3.39 (46.22)[a,b]	77.71±4.03 (64.74)[a,b]
AST (UI/l)	92.51±6.8	193.05±3.62 (108.68)[a]	127.51±3.74 (37.83)[a,b]	136.71±3.18 (47.78)[a,b]
Total bilirubin (mg/dl)	0.64±0.04	1.25±0.01 (95.31)[a]	0.814±0.02 (27.19)[a,b]	0.79±0.03 (23.44)[a,b]
Total protein (g/dl)	5.21±0.08	4.66±0.10 (–10.56)[a]	5.00±0.05 (–4.03)[b]	6.54±0.07 (25.53)[a,b]
Albumin (g/dl)	2.89±0.07	2.52±0.03 (–12.80)[a]	2.95±0.05 (2.08)[b]	3.11±0.03 (7.61)[a,b]
Glucose (mg/dl)	86.12±1.07	98.30±5.03 (14.14)[a]	83.33±4.80 (–3.24)[b]	84.41±4.13 (–1.99)[b]
Total cholesterol (mg/dl)	89.35±3.09	110.02±4.71 (23.13)[a]	88.10±3.72 (–1.40)[b]	92.58±3.09 (3.61)[b]
Triacylglycerols (mg/dl)	53.27±1.69	86.91±5.52 (63.15)[a]	51.20±2.99 (–3.89)[b]	52.24±2.96 (–1.93)[b]

All data are represented as mean±SE; ALT, alanine transaminase; AST, aspartate transaminase; [a]Significant difference versus control group at $P<0.05$; [b]Significant difference versus thioacetamide intoxicated group at $P<0.05$. Numbers between brackets represent percent change from control group.

Figure 1



Micrographs of liver section from A): G (I) shows the normal architecture of the portal tract (arrow). At the wall of sinusoids, phagocytic irregular cells known as Von Kupffer cells are shown (arrowhead), B): G (II) shows nodular appearance of the liver lobule and bridging necrosis with accompanied mononuclear inflammatory cells (arrows). Also signs of vacuolar degeneration of hepatocytes with balloon cells (arrowhead) and focal proliferation of biliary ductules within the periportal tracts(green arrow) are visible, C): G (III) shows acute inflammatory infiltrate (arrow) within the periportal tract entering focally the liver lobule and vacuolar degeneration of single hepatocytes. Fibrotic septa are not seen, D): G (III) shows mild inflammation (arrow) but no fibrotic septa. Hepatocytes appear more or less like normal ones with small areas of hydropic degeneration, E): G (IV) shows mild inflammation (arrow) in portal and periportal tract without fibrotic septa. Hepatocytes appear more or less like normal ones, F): G (IV) shows hepatocytes appear more or less like normal ones (H&E, Scale bar: 20 μm).

showed occasional thin septa; may have portal expansion (Fig. 2e), and some liver sections showed no septa or rare thin septa; may have portal expansion or mild sinusoidal fibrosis (Fig. 2f). In addition, the fibrotic areas calculated from the examined liver sections showed a significant decrease ($P<0.05$) in comparison with the TA group (Table 3).

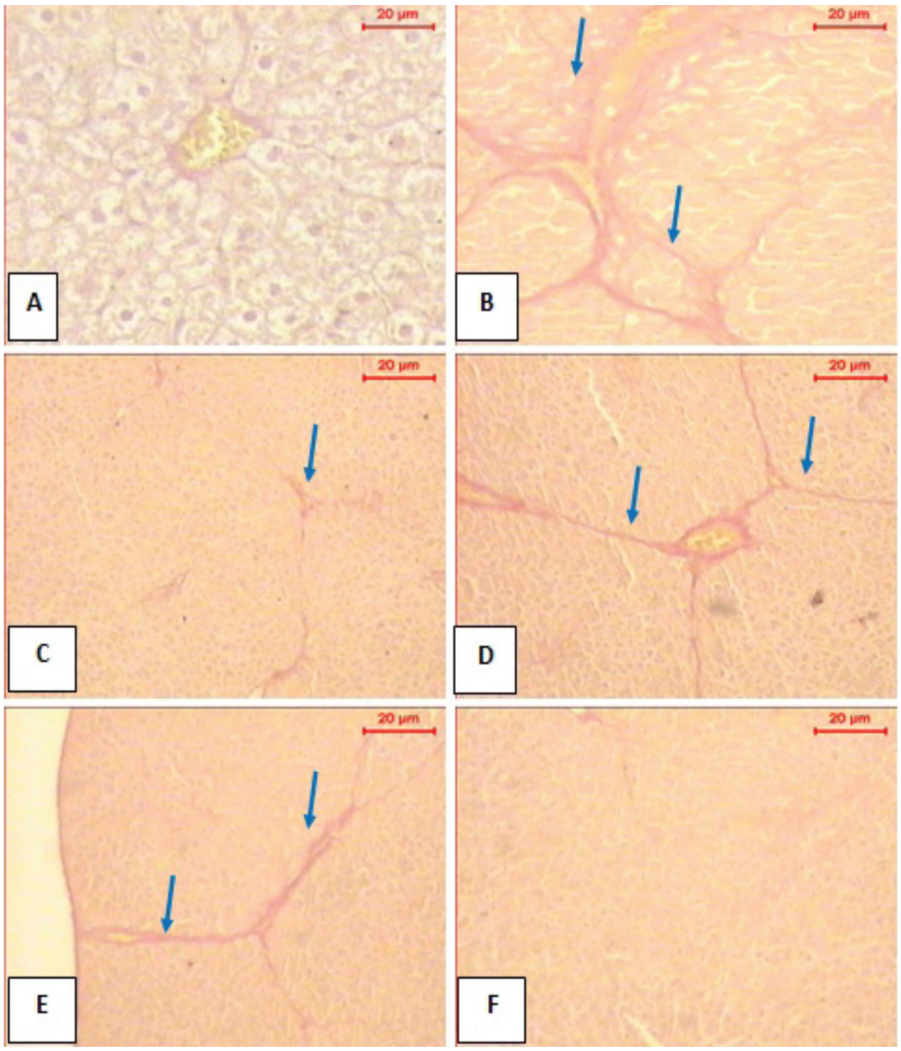
Correlations between liver galectin-3 level and other markers

The data presented in Table 4 exhibited that liver Gal-3 positively correlated with each of liver TGF-β1 ($P<0.01$), MDA ($P<0.05$), NO ($P<0.05$), and serum ALT and AST activities ($P<0.01$). Additionally, the results showed a negative correlation between liver Gal-3 and each of liver TAC ($P<0.01$) and catalase activity ($P<0.01$). The other markers exhibited insignificant correlation with liver Gal-3.

Discussion

Liver fibrosis, and in certain cirrhosis, have become major endpoints in patients with chronic liver diseases. Chronic liver disease reduces the regenerative capacity of liver tissues, which becomes insufficient; besides, apoptosis or necrosis of hepatic parenchymal cells becomes more favoured. This results in the deposition of ECM components and formation of scar [41]. Excessive formation of scar tissues distorts the vascular architecture of the liver tissue and may lead to liver dysfunction [2]. Therefore, an urgent need for an effective antifibrotic drug has appeared,

Figure 2



Micrographs of liver section from A): G (I) shows normal hepatocytes architecture and mild congestion within the periportal vein, B): G (II) shows intense fibrosis (arrows) dividing the liver lobule into the rounded contours or visible nodules, C): G (III) shows mild thin septa, D): G (III) shows red positive area that indicated moderate fibrosis within the periportal tract entering focally the liver lobule, E): G (IV) shows Occasional thin septa (arrows); may have portal expansion, F): G (IV) shows no septa or rare thin septa; may have portal expansion or mild sinusoidal fibrosis (Van Gieson stain, Scale bar: 20 µm).

Table 3 Fibrotic areas calculated from the examined rat liver sections of the different experimental groups

Groups	Parameters	
	Fibrotic area	%
Thioacetamide (group II)	1247±335	–
Thioacetamide+silymarin (group III)	388±14.7[a]	–68.88
Thioacetamide+black seed (group IV)	600±39.9[a]	–51.88

All data are represented as mean±SE; [a]Significant difference versus thioacetamide intoxicated group at $P<0.05$. %: percent change from thioacetamide intoxicated group.

a drug that enhances resolving fibrosis independent of the etiologic cause of fibrosis.

Until now there is no standard treatment or specific drug for liver fibrosis, but a reduction in liver injury events such as cessation of alcohol intake or successful viral hepatitis treatment can control fibrosis progression.

Table 4 Pearson's correlation between liver galectin-3 and each of liver antioxidant and oxidative stress parameters and serum transaminases

Parameters	*r*	*P*
TGF-β1	0.828	<0.01
TAC	–0.569	<0.01
Catalase	–0.618	<0.05
MDA	0.604	<0.05
NO	0.567	<0.05
ALT	0.730	<0.01
AST	0.805	<0.01

ALT, alanine transaminase; AST, aspartate transaminase; MDA, malondialdehyde; NO, nitric oxide; *P*, two-tailed test significance value; *r*, Pearson's correlation coefficient; TAC, total antioxidant capacity; TGF-β1, transforming growth factor-β1; Correlation is considered significant at $P<0.05$.

Nevertheless, these actions are often insufficient to avoid eventual progression to cirrhosis in the vast

majority of patients [5]. Recently, Gal-3 was found related to fibrogenesis in different organs. It is believed that Gal-3 has a vital role in TGF-β1-mediated activation of myofibroblasts to secrete ECM components, which is a key step in fibrogenesis [42–44].

Finding a suppressor of Gal-3 expression can help in the development of novel potential antifibrotic therapies. Natural products with known hepatoprotective and antifibrotic history are important candidates for finding a probable safe inhibitor for Gal-3 with minimum or no side effects. The present study aimed to investigate the effect of *N. sativa* seed (black seed) on Gal-3 level and the histological features of the liver in TA-induced liver injury of rats. We focused on the biochemical changes elicited in liver tissues including TGF-β1, TAC, catalase, MDA, NO, and serum liver function parameters, plasma glucose, and lipid profile, in addition to the histopathological changes in liver cells.

TA administration resulted in a significant elevation of liver Gal-3 and TGF-β1 levels in comparison with the control group. These results are in coincidence with Henderson *et al.* [42], MacKinnon *et al.* [43], and Li *et al.* [44], who reported Gal-3 elevation as a result of tissue injury in different body organs. This coincides with our histopathological results showing large fibrotic areas. Through activation of MAPK/MEK pathway, TA-induced oxidative stress can trigger activation of activator protein-1 (AP-1), leading to the upregulation of Gal-3 expression [45]. TA-induced oxidative stress causes hepatocellular injury and necrosis. These events activate macrophages, Kupffer cells, and cholangiocytes; resulting in the secretion of TGF-β1 and other cytokines [46,47].

Treatment with silymarin and black seed antagonized TA toxic effect on liver Gal-3 and TGF-β1 levels, and the levels of the two parameters in both groups III and IV were close. Silymarin effect on Gal-3 may be due to its interference with and downregulation of each of ERK1/2 induced activation of c-Jun/AP-1 and transcription factor NF-κB, which are two transcriptional factors regulating Gal-3 expression [48]. In addition to AP-1 and NF-κB, silymarin inhibits hypoxia-inducible factor 1. All these transcriptional factors regulate TGF-β1 gene expression and were reported to be inhibited by silymarin [49,50].

The inhibitory effect of black seed on both of Gal-3 and TGF-β1 level may be related to thymoquinone, the most abundant component of *N. sativa* seed. Thymoquinone has inhibitory effect on the activation of the transcriptional factor NF-κB which regulates Gal-3 and TGF-β1 gene expression [51–53].

The oxidative stress induced by TA active sulfur metabolite ($TASO_2$) resulted in significant depression of liver TAC level and catalase activity besides significant elevation in liver MDA and NO levels. These results are in accordance with Chen *et al.* [54], Song and Chen [55], and Nehar and Kumari [56]. Chronic oxidative stress leads to high consumption rate of antioxidant enzymes and molecules that exceeded biosynthesis rate. $TASO_2$ attacks cell components especially cell membrane lipids causing lipid peroxidation leading finally to the formation of MDA [57]. Oxidative stress stimulates immune cells like mast cells to produce and secrete cytokines that activate nitric oxide synthase (iNOS) expression. iNOS is responsible for NO production in macrophages [54].

Both silymarin and black seed exhibited a protective effect against TA-induced oxidative damage, represented in significant higher liver TAC level, catalase activity, and significant lower liver MDA and NO levels in groups III and IV when compared with group II. These results are in accordance with Jia *et al.* [58], Freitag *et al.* [59], Ismail *et al.* [60], and Umar *et al.* [61]. Silymarin contains silybin and several flavonoids with antioxidant properties. It inhibits lipid peroxidation caused by free radicals; thus decreases MDA production which results in preventing cell injury leading to a decreased level of NO production. Silymarin also inhibits iNOS expression [58,62]. Meanwhile black seed contains many phenolic compounds, mostly thymoquinone, p-cymene, and carvacrol which have been reported to have antioxidant properties. These compounds act as a shelter protecting against lipid peroxidation and preventing high rate of cellular antioxidants consumption and depletion [63].

The rats intoxicated with TA showed significant increased serum ALT and AST activities and total bilirubin level in comparison with control rats. These results agree with Bludovska *et al.* [64] and Amin *et al.* [65]. $TASO_2$ attacks lipids of cell membranes, leading to damage in hepatocytes cell membranes resulting in the leakage of intracellular components outside the cell [63]. TA induces impairment in the liver function of uptaking biliurubin from blood due to hepatocellular necrosis and bile duct damage [65].

Treatment with silymarin and black seed significantly decreased serum ALT and AST activities and total

bilirubin level in comparison with group II. The results were similar in the two treated groups III and IV. These results are in accordance with Eminzade *et al.* [66], Chen *et al.* [67], Nehar and Kumari [56], Saricicek *et al.* [68], and Elkhateeb *et al.* [69]. The antioxidant properties of both silymarin and black seed ameliorate TA-induced oxidative damage on hepatocytes and bile ducts and maintain cell membrane integrity [67,70].

TA administration resulted in a significant depression of serum total protein, albumin, and significant elevation of serum total cholesterol and triacylglycerol levels in comparison with the control group. These results are in accordance with Khalaf *et al.* [71], Mustafa *et al.* [72], and Abdalla *et al.* [73]. TA intoxication results in severe damage to polyribosomes found on the endoplasmic reticulum of hepatocytes, leading to depression in protein synthesis [73]. The long period of administration of TA leads to necrosis of a great number of hepatocytes and exhausts the regenerative ability of the liver tissues. As a result, great loss occurs in liver functions of protein synthesis and uptaking total cholesterol and triacylglycerols from the blood, along with impairments in protein and lipid metabolism [64,65,73].

Significant increase was found in serum total protein, albumin and significant decrease of serum total cholesterol and triacylglycerol levels in the groups treated with silymarin and black seed when compared with group II. These results agree with Eminzade *et al.* [66], Essawy *et al.* [74], Cacciapuoti *et al.* [75], and Saricicek *et al.* [68]. These results probably are due to the antioxidant activities of both silymarin and black seed, which shelter hepatocytes from excessive oxidative damage induced by TA administration [68,70]. This preserved the functionality of a greater portion of liver than in case of group II. Moreover, black seed has cholerectic activity that functions through reducing cholesterol biosynthesis by hepatocytes or by decreasing cholesterol fractional absorption by the small intestine [76]. It is worth mentioning that serum total protein level of group IV increased significantly in comparison with the control group in coincidence with Saricicek *et al.* [68]. This may be due to the increased rate of protein synthesis induced by black seed during healing and regeneration of injured hepatocytes.

Fasting plasma glucose decreases than the normal range only in cases of hepatic failure due to severe damage as in the last stage of cirrhosis [65], and in the present study we did not reach that stage; so fasting blood glucose level remained in the normal range in all the experimental groups.

TA administration resulted in great damage to the rat liver represented by nodular appearance of the liver lobule, bridging necrosis accompanied with vacuolar degeneration of hepatocytes. These results are parallel with De David *et al.* [77] and coincide with Chen *et al.* [67]. This also comes in line with our biochemical analyses results. TA is a hepatotoxin that causes centrilobular necrosis; chronic liver injury stimulates the replacement of parenchymal cells by resident ECM which forms fibrosis [2].

Treatment with silymarin and black seed resulted in an improvement in the liver architecture represented in reduced areas of fibrosis in comparison with group II, minor fibrotic septa and mild inflammation. These results are in agreement with Chen *et al.* [67], Essawy *et al.* [74], and Jaswal and Shukla [70] and confirm our biochemical analyses results.

The correlation we found between Gal-3 and TGF-β1 may be related to the direct effect of Gal-3 on the activation of TGF-β1expresion pathway. Gal-3 stimulates macrophages to secrete TNF-α, which induces activation of the transcriptional factor TN-κB [78,79], which in turn induces expression of TGF-β1. Regarding the correlation between liver Gal-3 and liver TAC, MDA, NO levels, catalase, and serum ALT and AST activities, this relation can be related to a common factor caused the elevation which is oxidative stress.

Conclusion

In conclusion, the present findings provide clear experimental evidence for the inhibitory effect of black seed on Gal-3 level in the liver of rats after TA-induced injury. This inhibitory effect may be related to the direct effect of black seed on the transcriptional factors NF-κB and AP-1 which are both responsible for Gal-3 expression. Besides, there is an indirect role of black seed through its antioxidant properties fighting against oxidative stress caused after TA administration. Gal-3 showed significant positive correlations with other biomarkers for liver disease. These results suggest Gal-3 to be a reliable and significant indicator for screening liver disease progression.

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Conflicts of interest
There are no conflicts of interest.

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