Galectin-3 and fibrosis levels in experimental liver injury treated with honey alone or mixed with *Nigella sativa* seed

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Background/aim
Galectin-3 (Gal-3) is a multifunctional protein that plays an important role in many biological processes and is linked with fibrogenesis. The aim of this study was to evaluate the effect of honey alone or mixed with *Nigella sativa* seed (black seed), as hepatoprotective natural remedies on galactin-3 and liver fibrogenesis induced by thioacetamide (TA) in rats.
Materials and methods
This study was performed on 60 male rats divided into six groups (groups I–VI), with 10 rats each. Group I served as a normal control, groups from II–VI were intoxicated by TA, whereas the groups from III to VI were treated by silymarin, honey, black seed, and honey mixtures, respectively. Levels of liver Gal-3, transforming growth factor β1 (TGF-β1), oxidative stress markers, and serum liver function parameters were determined. Computerized image analysis was used to obtain quantitative measurement of liver fibrotic areas.
Results
Levels of liver Gal-3, TGF-β1, oxidative stress markers, serum liver transaminases, and fibrotic areas showed significant elevations in TA treated as compared with control. Treatment with silymarin and honey alone or mixed with black seed resulted in lowering liver Gal-3, TGF-β1 levels, and fibrotic areas and improved liver functions and antioxidant status. The mixture doses showed greater effect than honey against TA toxic effects on liver.
Conclusion
Honey either alone or mixed with black seed reduced Gal-3 level, reduced fibrosis propagation, and ameliorated the toxic effect of TA on liver; moreover, the mixture had greater effect than using honey alone and was closer to silymarin effect.

Keywords:
galectin-3, honey, liver fibrosis, *Nigella sativa* seed, thioacetamide

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Introduction

Liver diseases, including hepatitis with its types, cirrhosis, hepatocellular carcinoma, fatty liver, and non-alcoholic steatohepatitis, have affected many people around the world [1]. Chronic liver tissue injury causes chronic inflammation, which leads to formation of scar tissue (fibrosis), loss of tissue architecture, and finally, organ failure [2]. Fibrosis and, in certain cases, cirrhosis, represent the final end point of prolonged liver injury and are considered as the leading causes of morbidity and mortality [3].

Many trials have been carried out for producing an effective treatment for fibrosis; until now, no effective drug for resolving fibrosis has been produced. In these trials, chemical agents were used to inhibit myofibroblast activation pathways and extracellular matrix (ECM) production. These approaches worked well in tissue culture and in some rodent models of liver fibrosis but are still risky owing to the adverse effects [4,5].

Galectins are a subgroup of animal lectins. They have carbohydrate-recognition domain by which they can bind β-galactosides. Each galectin prefers binding to an individual species of carbohydrates. Galectins are distributed inside cell nucleus, cytoplasm, ECM, and on cell surface [6]. Galectin-3 (Gal-3) is a multifunctional protein. It plays an important role in many biological processes. It has been reported to be involved in cell adhesion, cell signaling, cell proliferation, apoptosis and angiogenesis, immune reactions, and fibrogenesis [7].

The soluble part of Gal-3 is responsible for activation of myofibroblast, leading to collagen secretion in ECM followed by fibrogenesis. In addition, development of fibrosis in a variety of organs, such as the liver, kidney, gut and heart, has been related to Gal-3 expression [7,8]. Gal-3 participates in thioacetamide (TA) acute liver injury through stimulating production of monocyte chemoattractant protein-1 and transforming growth factor-β1 (TGF-β1) [9]. TGF-β1 is a cytokine that enhances fibrosis in the biological systems. TGF-β1 induces the production of ECM components. Hepatic stellate cells are TGF-β1 main production sites in liver [10]. During inflammation, TGF-β1 induces transformation of hepatic stellate cells into activated myofibroblasts which produce and secrete ECM components. Accumulation of ECM leads to scar formation [8,9].

Honey is a natural product of honeybees that has many nutritional benefits. It has been used for many centuries in traditional medicine [11]. It consists mainly of the sugars fructose (38%) and glucose (31%) and minor components such as flavonoids, phenolic acids, carotenoid-like substances, and some enzymes, such as catalase [12–14]. Honey was reported to have medicinal properties, such as antibacterial, antioxidant, anti-inflammatory, hepatoprotective, antitumor, and antiviral [11–15].

Nigella sativa (black seed) is a natural food that has been involved in Eastern Traditional Medicine. It is reported to have various medicinal characteristics such as immunomodulatory, renal-protective and hepatoprotective, anti-inflammatory, and antioxidant properties. Its hepatoprotective role against toxic chemicals such as carbon tetrachloride and heavy metals has been verified [16–18].

TA is a hepatotoxin that induces centrilobular necrosis in the liver. TA serves as a widely used model for induction of acute and chronic liver injury and fibrosis. TA induces micronodular cirrhosis, which closely resembles what occurs in human liver cirrhosis [19,20].

Silymarin is a common drug used in the treatment of hepatic dysfunction. Silymarin contains a mixture of flavolignans and organic compounds, which have antioxidant properties, and is extracted from the plant *Silybum marianum* [21]. It acts as hepatoprotective agent against hepatic injury induced by oxidative stress and toxic substances [22].

This study aimed to evaluate the effect of honey alone or mixed with black seed on Gal-3 level in rat liver injury induced by TA to compare their effect with that of silymarin. Additionally, the correlations between Gal-3 level and other biochemical markers for liver injury, as well as histopathological changes of liver, were investigated.

Materials and methods

Honey, black seed, and silymarin

Honey and black seed were purchased from the Ministry of Agriculture products selling port, Giza, Egypt. Both honey and black seed were freshly prepared before every administration. A volume of 1300 mg of honey was diluted by water (1 : 1) (w/w) before usage. A volume of 800 mg of black seed was washed, dried, ground, and then suspended in water before use. Silymarin was obtained from the pharmacy in form of powder sachets produced by SEDICO Pharmaceutical Co. (6 October City, Egypt). Each sachet contains 140 mg silymarin. It was freshly prepared and administered by dissolving the content of each sachet in water (50 ml).

Animals and ethical consideration

A total of 60 male rats, weighing 90–110 g each, were obtained from National Research Centre Lab House. The animals were maintained under standardized environmental conditions at 12 h light/dark cycle and 25±1°C. Rats were fed on basal diet [23,24], and water was supplied *ad libitum*. All experimental procedures were performed according to guidelines of the Institutional Committee of Animal's Care and Use, National Research Centre, Egypt. The study protocol was approved by the Ethical Committee Board of the National Research Centre, Cairo, Egypt.

Experimental design

The experiment took 7 weeks [25], and rats were divided into six groups (10 rats each), as follows:

(1) Group I: served as normal control and received saline intraperitoneally (0.01 mg/kg b. wt).
(2) Group II: TA group, which was injected intraperitoneally with 200 mg/kg b. wt. of TA two times per week [25].
(3) Group III: silymarin and TA group, which was intoxicated with TA as in group II and daily received 50 mg/kg b. wt. of silymarin orally.
(4) Group IV: honey and TA group, which was intoxicated with TA as in group II and daily received 50 mg/kg b. wt. of honey orally [26].
(5) Group V: mixture 1 group, which was intoxicated with TA as in group II and daily received 50 mg/

Kg b. wt. of honey and black seed mixture (25 mg honey plus 25 mg black seed) orally.

(6) Group VI: mixture 2 group, which was administered with TA as in group II and daily received 100 mg/kg b. wt. of honey and black seed mixture (50 mg honey plus 50 mg black seed) orally.

As the mixture was not used before in literature, we used mixture doses that contain honey amount half or equal to the dose mentioned in the honey-administered group (group IV) according to Khadr *et al.* [26].

Blood and liver samples

At the end of the experiment, blood samples were collected in clean dry test tubes after 16 hours fasting using the orbital sinus technique of Sanford [27]. Samples were left to clot and then centrifuged at 3000 rpm for ten minutes. The clear supernatant serum was then separated and frozen at −20°C for the biochemical analysis. 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 examination.

Biochemical analyses methods

Liver Gal-3 and TGF-β1 levels were determined by ELISA technique using kit purchased from Sun Red Biotechnology Co. (Shanghai, China), according to the method described by the manufacturer using Stat Fax 2100 Microplate Reader, Awareness Technologies Inc. (Palm City, Florida, USA). Liver total antioxidant capacity (TAC), catalase, malondialdehyde (MDA), and nitric oxide (NO) were estimated using commercial kits purchased from Biodiagnostic Co., Giza, Egypt, based on the methods described by Koracevic *et al.* [28], Aebi [29], Satoh [30], and Montgomery, and Dymock [31] respectively.

Serum alanine (ALT) and aspartate (AST) transaminases were estimated using kits of Vitro Scient Co. (Hannover, Germany), based on the methods described by Bergmeyer *et al.* [32] and Henry *et al.* [33], respectively. Serum total bilirubin was determined according to the method of Walter and Gerade [34], using kit of Biodiagnostic Co. (Giza, Egypt). Serum total protein, albumin, and plasma glucose were estimated using kits purchased from Spectrum Co., based on the methods described by Cannon *et al.* [35], Doumas *et al.* [36], and Howanitz *et al.* [37], respectively. Serum total cholesterol was estimated by the method of Allain *et al.* [38], whereas triacylglycerol was estimated by the method of Fossati and Prencipe [39], using kits of Vitro Scient Co..

Histopathological examination and image analysis

The liver of rats of different groups was removed and fixed in 10% formal saline. Overall, 5-μm-thick paraffin sections were stained with hematoxylin and eosin dyes [40] 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 was performed using method of Bancroft and Cook [41]. Quantitative measurement of fibrotic areas was achieved by using computerized image analysis (Leica Qwin 500) in Pathology Department, National Research Centre. Ten nonoverlapping fields per rat liver slide at a final magnification of 100× were randomly selected, and Van Gieson's stain-positive areas were calculated, and 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 analysis of variance test. *P* values less than 0.05 were considered statistically significant [42].

Results

Biochemical results

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

Table 1 Galectin-3, TGF-β1, antioxidant, and oxidative stress parameters in liver cells of the experimental rat groups

Groups	Parameters					
	Galectin-3 (ng/mg tissue)	TGF-β1 (ng/mg tissue)	TAC (mmol/mg tissue)	Catalase (U/g tissue)	MDA (nmol/g tissue)	NO (μmol/g tissue)
Control (group I)	2.71±0.2[a]	37.85±4.4[a]	12.16±0.7[a]	3611±125[a]	1259±177[a]	1121±49[a]
Thioacetamide (group II)	4.86±0.74[b]	88.25±9.3[b]	3.82±0.4[b]	2244±183[b]	6214±458[b]	3671±285[b]
Thioacetamide+silymarin (group III)	3.56±0.2[c]	58.18±5.7[c]	10.13±0.9[a]	3149±296[a,c]	1942±246[a,c]	1533±115[a,c]
Thioacetamide+honey (group IV)	3.90±0.23[bc]	53.35±6.2[c]	5.76±0.5[c]	2954±240[d]	3433±298[d]	2350±110[d]
Thioacetamide+mix. 50 mg (group V)	3.23±0.11[a,c]	52.37±4.5[c]	8.08±0.8[d]	2975±138[d]	2829±242[e]	2080±147[d]
Thioacetamide+mix. 100 mg (group VI)	3.46±0.25[a,c]	49.97±5.5[a,c]	8.8±0.89[e]	3058±294[c,d]	2166±250[c]	1711±189[c]

All data are represented as mean±SE. MDA, malondialdehyde; NO, nitric oxide; TAC, total antioxidant capacity; TGF-β1, transforming growth factor β1. Values with different letters (a, b, c, d, e) are significant at $P<0.05$ using analysis of variance test.

Table 2 Serum levels of liver function parameters in the experimental rat groups

Groups	Parameters				
	ALT (U/l)	AST (U/l)	Total bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
Control (group I)	44.59±5.7[a]	88.15±7.3[a]	0.54±0.04[a]	5.05±0.3[a]	2.97±0.2[a]
Thioacetamide (group II)	181.9±22.4[b]	212.5±18.4[b]	1.34±0.08[b]	3.94±0.7[b]	2.36±0.3[b]
Thioacetamide+Silymarin (group III)	61.4±7.2[c]	120.8±15.6[c]	0.81±0.07[c]	4.9±0.4[a]	2.99±0.4[a,c]
Thioacetamide+honey (group IV)	92.4±9.5[d]	131.8±14.9[c]	0.82±0.1[c]	6.28±0.9[c]	3.12±0.4[c,d,e]
Thioacetamide+mix. 50 mg (group V)	76.6±6.8[c]	114.2±10.5[c]	0.80±0.09[c]	6.34±0.8[c]	3.01±0.3[a,d]
Thioacetamide+mix. 100 mg (group VI)	72.4±8.8[c]	113.6±14[c]	0.76±0.09[c]	6.84±0.8[d]	3.26±0.4[e]

All data are represented as mean±SE. ALT, alanine transaminase; AST, aspartate transaminase. Values with different letters (a, b, c, d, e) are significant at $P<0.05$ using analysis of variance test.

Table 3 Plasma glucose, serum total cholesterol, and triacylglycerols levels in the experimental rat groups

Groups	Parameters		
	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triacylglycerols (mg/dl)
Control (group I)	81.85±3.5[a]	86.18±4.8[a]	49.7±2.9[a]
Thioacetamide (group II)	95.1±7[b]	115±6.6[b]	91.2±7.3[b]
Thioacetamide+silymarin (group III)	81.7±6[a]	87.3±6.1[a]	55.8±5.5[a]
Thioacetamide+honey (group IV)	85.4±7.8[a]	88.4±8[a]	54±5.1[a]
Thioacetamide+mix. 50 mg (group V)	91.1±8.5[a,b]	87.5±7.9[a]	58.5±7.9[a]
Thioacetamide+mix. 100 mg (group VI)	91.5±9.4[a,b]	54.8±5[a]	53.9±5.7[a]

All data are represented as mean±SE. Values with different letters (a, b) are significant at $P<0.05$ using analysis of variance test.

with group I, whereas both of them decreased significantly ($P<0.05$) in all treated groups when compared with group II.

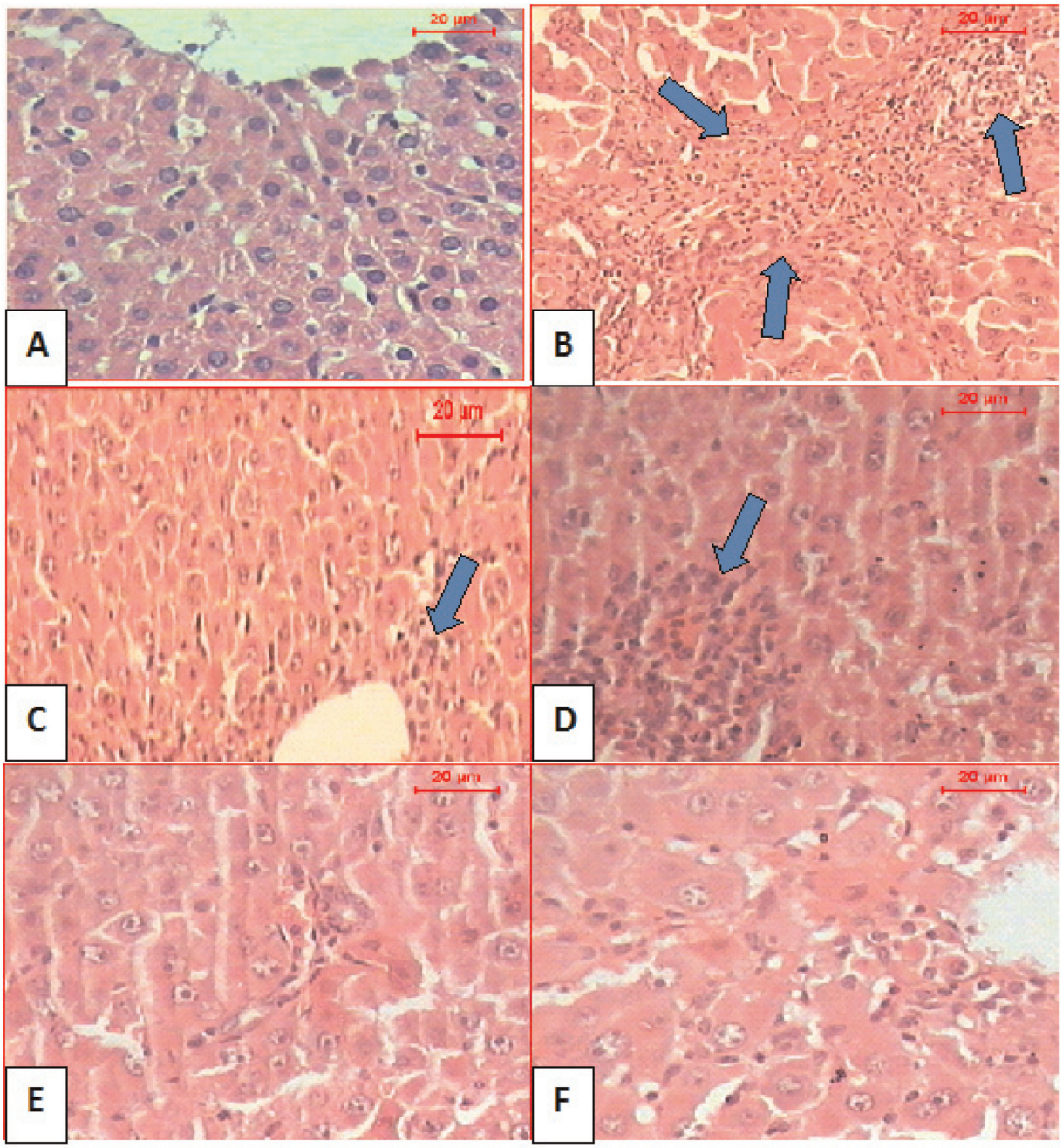
Table 2 shows the results of serum liver function parameters. It was found that each of serum ALT and AST activities and total bilirubin level increased significantly ($P<0.05$) in all groups when compared with group I, whereas they decreased significantly ($P<0.05$) in all treated groups when compared 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 groups IV–VI, except group V, which showed insignificant increase in serum albumin level. Meanwhile, both serum total protein and albumin levels increased significantly ($P<0.05$) in all treated groups when compared with group II.

Regarding serum total cholesterol, triacylglycerols, and plasma glucose, their levels increased significantly ($P<0.05$) in group II in comparison with group I. Moreover, the three parameters levels decreased significantly ($P<0.05$) in all treated groups when compared with group II, except groups V and VI, which showed insignificant decrease in glucose level, as shown in Table 3.

Histopathological examinations and image analysis

Histopathological results of control rats showed normal structure of the hepatic lobules (Fig. 1a).

Figure 1

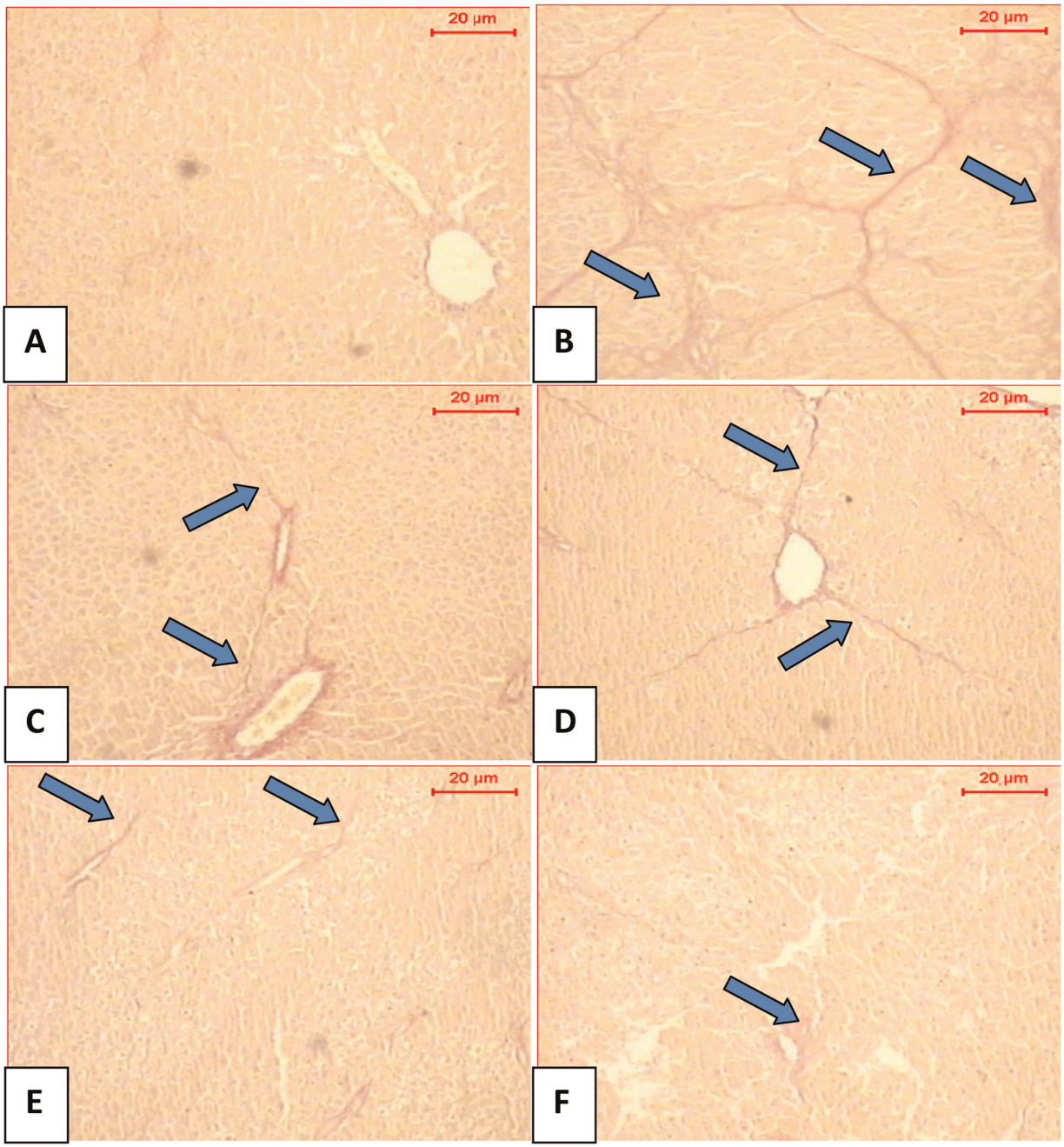


Micrographs of liver section from (a): control group shows the normal architecture of the portal tract. (b) Thioacetamide intoxicated group (group II) shows nodular appearance of the liver lobule and bridging necrosis with accompanied mononuclear inflammatory cells (arrows). (c) Thioacetamide and silymarin co-administered group (group III) shows mild inflammation (arrow) but no fibrotic septa. Hepatocytes appear more or less like normal ones with small areas of vacuolar degeneration. (d) Thioacetamide and honey co-administered group (group IV) shows mild inflammation (arrow) in portal and periportal tract without fibrotic septa. Hepatocytes appear more or less like normal ones. (e) Thioacetamide and mixture 50 mg/kg b. wt. co-administered group (group V) shows portal tract with no inflammation and narrow fibrotic septa. Hepatocytes appear more or less like normal one. (f) Thioacetamide and mixture 100 mg/kg b. wt. co-administered group (group VI) shows normal hepatic lobule and no inflammation. Hepatocytes appear more or less like normal one (hematoxylin and eosin, scale bar: 20 µm).

Van Gieson's staining of liver of normal control rats showed normal hepatic architecture (Fig. 2a).

Meanwhile, liver sections from TA-intoxicated rats showed nodular appearance of the liver lobules, bridging necrosis accompanied with a large number of mononuclear inflammatory cells, and signs of vacuolar degeneration of hepatocytes with balloon cells (Fig. 1b). In addition, Van Gieson's staining of group II liver sections revealed intense fibrosis, dividing the liver lobules into the rounded contours or visible nodules (Fig. 2b). The mean of fibrotic areas found in the examined rat liver sections of group II was 1247±335 μm^2 (Table 4).

Figure 2



Micrographs of liver sections from (a): control group (group I) shows normal hepatocytes architecture. (b) Thioacetamide intoxicated group (group II) shows intense fibrosis (arrows) dividing the liver lobule into the rounded contours or visible nodules (arrows). (c) Thioacetamide and silymarin co-administered group (group III) shows mild thin septa (arrows). (d) Thioacetamide and honey co-administered group (group IV) shows mild fibrosis (arrows) within the periportal tract entering focally the liver lobule (arrow). (e) Thioacetamide and mixture 50 mg/kg b. wt. co-administered group (group V) shows no septa or rare thin septum (arrows); may have portal expansion. (f) Thioacetamide and mixture 100 mg/kg b. wt. co-administered group (group VI) shows no septa or a rare thin septum; may have portal expansion (arrow) (Van Gieson's stain, Scale bar: 20 µm).

However, rat liver sections of TA and silymarin co-administered group exhibited acute inflammatory infiltrate within the periportal tract entering focally the liver lobule. Hepatocytes appeared more or less like normal one with small vacuolar degeneration of single hepatocytes (Fig. 1c). By using Van Gieson's stain, liver sections of this group showed moderate thin septa that indicated mild fibrosis within the periportal tract entering focally the liver lobules (Fig. 2c). In addition, the fibrotic areas calculated from the examined liver sections showed a significant decrease ($P<0.05$) in comparison with group II (Table 4).

The histopathological study of liver sections from TA and honey co-administered group (group IV) showed preserved hepatocytes and small area of necrosis

Table 4 Fibrotic areas calculated from the examined rat liver sections of the different experimental groups

Group	Parameter Liver fibrotic area
Thioacetamide (group II)	1311±125[a]
Thioacetamide+silymarin (group III)	370±29.7[b]
Thioacetamide+honey (group IV)	660±75.6[c]
Thioacetamide+mix. 50 mg (group V)	555±63.7[d]
Thioacetamide+mix. 100 mg (group VI)	549±36.8[d]

All data are represented as mean±SE. Values with different letters (a, b, c, d) are significant at $P<0.05$ using analysis of variance test.

(Fig. 1d). However, Van Gieson-stained liver sections of the same group revealed moderate thin septa and mild fibrosis within the periportal tract entering focally 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 group II (Table 4).

Meanwhile, portal tract with no inflammation and narrow fibrotic septa appeared in liver sections from TA and mixture 50 mg/kg b. wt. co-administered group (group V). Moreover, hepatocytes appeared more or less like normal ones as shown in Fig. 1e. The liver sections stained by Van Gieson's stain revealed no septa or rare thin septa— may have portal expansion (Fig. 2e). The fibrotic areas calculated from the examined liver sections showed a significant decrease ($P<0.05$) in comparison with group II (Table 4).

Finally, liver sections from TA and mixture 100 mg/kg b. wt. co-administered group (group VI) exhibited normal hepatic lobule with rare mild fibrotic septa and no inflammation. Hepatocytes appeared more or less like normal ones (Fig. 1f). Van Gieson's staining showed occasional thin septa—may have portal expansion, as shown in Fig. 2f. Additionally, the fibrotic areas calculated from the examined liver sections showed a significant decrease ($P<0.05$) when compared with group II (Table 4).

Correlations between liver galectin-3 level and other markers

The data presented in Table 5 show that liver Gal-3 was positively correlated with each of liver TGF-β1 ($P<0.01$), MDA ($P<0.05$), and NO ($P<0.05$) levels; serum ALT and AST activities ($P<0.01$); and liver fibrotic area ($P<0.05$). Meanwhile, Gal-3 was negatively correlated with each of liver TAC level ($P<0.05$) and catalase activity ($P<0.05$). Finally, there was insignificant correlation between liver Gal-3 and the other markers.

Table 5 Pearson's correlation between liver galectin-3 and each of liver antioxidant and oxidative stress parameters, serum transaminases and liver fibrotic area

Parameters	*r*	*P*
TGF-β1	0.723	<0.01
TAC	–0.526	<0.05
Catalase	–0.538	<0.05
MDA	0.547	<0.05
NO	0.536	<0.05
ALT	0.738	<0.01
AST	0.832	<0.01
Fibrotic area	0.612	<0.05

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 $P<0.05$.

Discussion

Chronic liver disease usually leads to fibrosis and cirrhosis in late stages of disease. It results in reduction of the regeneration capacity of liver tissue, which becomes insufficient, and on the other side, it enhances apoptosis or necrosis of hepatic parenchymal cells. Subsequent to these events, deposition of ECM components and formation of scar occur in liver tissue [43]. Distortion of liver tissue vascular architecture occurs owing to excess formation of scars and can lead to liver dysfunction [2]. Therefore, there is an emerging need for effective antifibrotic drug that acts on resolving fibrosis independently of the etiologic cause of fibrosis.

Until now, there is no standard treatment or specific drug for liver fibrosis. However, reduction of fibrosis progression depends on removal of the etiologic cause, such as successful viral hepatitis treatment or avoiding alcohol intake. Nevertheless, these actions are often insufficient to prevent progression to cirrhosis in most patients [5]. Recently, Gal-3) was found to be linked to fibrogenesis in many organs. It was found to be involved in TGF-β1-mediated activation of myofibroblasts. This stimulates them to secrete ECM components and is considered a key step in fibrogenesis [44–46].

So, inhibition of Gal-3 seems a promising antifibrotic potential therapy. Natural extracts of plants or animal products, with known hepatoprotective and antifibrotic history, represent sustainable sources for finding a safe inhibitor for Gal-3, with minimum or no adverse effects. We previously studied the effect of black seed on Gal-3 level and liver fibrogenesis in TA-induced liver injury in rats in Salem *et al.* [47]. In this work, the effect of administration of honey alone or

mixed with black seed on Gal-3 level in liver injured rats has been investigated, besides its effect on histological features of liver. This has been achieved through the determination of biochemical changes in liver tissues including, TGF-β1, TAC, catalase, MDA, NO, serum liver function parameters, glucose, and lipid profile, in addition to examining histopathological changes in liver tissue.

Administration of TA led to a significant increase in liver Gal-3 and TGF-β1 levels when compared with control group. These results agree with Henderson *et al.* [44], MacKinnon *et al.* [45], and Li *et al.* [46], who reported Gal-3 level elevation after tissue injury in different organs. This agrees with our histopathological results, which revealed presence of large fibrotic areas in liver. TA administration induces oxidative stress, which activates activator protein-1 (AP-1) through MAPK/MEK pathway, subsequently leading to upregulation of Gal-3 expression [48]. Additionally, the induced oxidative stress causes hepatocellular injury and necrosis, which in turn stimulate macrophages, Kupffer cells, and cholangiocytes to release TGF-β1 and other cytokines [49,50].

Each of silymarin and honey alone or mixed with black seed reduced the effect of TA administration on liver Gal-3 and TGF-β1 levels. Silymarin interferes with 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 [51]. This could be the reason behind the reducing effect of silymarin on Gal-3 level. Additionally, silymarin inhibits hypoxia inducible factor 1, which besides AP-1 and NF-κB are three transcriptional factors that regulate TGF-β1 gene expression [52,53].

The inhibitory effect of honey on both of Gal-3 and TGF-β1 levels may be related to quercetin and kaempferol, which are two important components of honey. Both of them have inhibitory effect on activation of MAPK-dependent pathways of the transcriptional factors NF-κB and AP-1 [11,54]. Both of NF-κB and AP-1 regulate Gal-3 and TGF-β1 gene expressions as mentioned previously. On the contrary, black seed inhibitory effect can be linked to thymoquinone, the most abundant constituent of black seed, which inhibits the activation of the transcriptional factor NF-κB, which in turn inhibits Gal-3 and TGF-β1 genes expression; however, there is no link found between other polyphenolic constituents of black seed and Gal-3 level [55–57].

Thioacetamide active sulfur metabolite ($TASO_2$) starts oxidative stress cascade, which resulted in significant depression of liver TAC level and catalase activity besides significant elevation in liver MDA and NO levels. These results agree with Chen *et al.* [58] and Song and Chen [59]. Prolonged induced oxidative stress leads to high consumption of antioxidant enzymes and molecules at a rate exceeding the biosynthesis rate. $TASO_2$ attacks cell components and triggers lipid peroxidation of cell membrane lipids, leading finally to production of MDA [60]. The induced oxidative stress stimulated immune cells to secrete cytokines that activate expression of nitric oxide synthase (iNOS), which is responsible for NO production in macrophages [58].

Treatment with each of silymarin and honey alone or mixed with black seed sheltered the cells from TA-induced oxidative damage. This was shown clearly through significant higher liver TAC level, catalase activity, and significant lower levels of liver MDA and NO in the treated groups when compared with group II. These results are parallel with Freitag *et al.* [61], Wang *et al.* [62], and Ismail *et al.* [63]. Silymarin contains mainly flavonoids, including silybins, isosilybins, silychristin, and silydianin, which have antioxidant properties. They inhibit $TASO_2$-induced lipid peroxidation through providing electrons that stabilize free radicals, which in turn decreases MDA production. This leads to prevention of cell injury and decreases the level of NO production. This is besides the inhibitory effect of silymarin on iNOS gene expression, which decreases NO synthesis [61].

Honey contains various phenolic compounds (daidzein, benzoic acid, and cinnamic acid), flavones (quercetin, kaempferol, and genistein), and catalase enzyme, and also black seed has phenolic components like thymoquinone, p-cymene, and carvacrol, which had great effects in protection against oxidative stress [12,16]. These compounds protect the cells from lipid peroxidation and reduce the rate of cellular antioxidants consumption. They stabilize $TASO_2$ and other free radicals or active oxygen compounds by providing electrons, so they stop the free radical chain reaction of cell and organelles' membrane lipids [16,64].

TA administration resulted in significant increase of serum ALT and AST activities and total bilirubin level in comparison with control rats. These results are in accordance with Bludovska *et al.* [65] and Amin *et al.* [66]. $TASO_2$-induced lipid peroxidation, leading to

severe damage in hepatocytes cell membranes, which resulted in leakage of intracellular components to outside the cell [65]. In addition, TA administration causes hepatocellular necrosis and bile duct damage, which leads to impairment in liver function of uptaking bilirubin from blood [67].

Administration of silymarin and honey alone or mixed with black seed resulted in a significant decrease in serum ALT and AST activities and total bilirubin level in comparison with group II. These results coincide with Wang *et al*. [62], Chen *et al*. [68], and Saricicek *et al*. [69]. Because of their antioxidant properties, each of silymarin and honey alone or mixed with black seed relieves TA-induced oxidative stress on hepatocytes and bile ducts and maintains cell membrane integrity [68,69].

The rats intoxicated with TA exhibited significant decrease in serum total protein and albumin levels, and a significant increase in serum total cholesterol and triacylglycerols levels in comparison with control group. These results are parallel with Khalaf *et al.* [70], Mustafa *et al*. [67], and Abdalla *et al*. [71]. TA causes severe damage to the polyribosomes found on endoplasmic reticulum of hepatocytes, leading to impairment in protein synthesis [71]. Chronic administration of TA causes the necrosis of a large number of hepatocytes and exhausts their regeneration capacity. This leads to great loss occurring in liver functions of protein synthesis and uptaking total cholesterol and triacylglycerols from blood, along with impairments in protein and lipid metabolism [65,66,71].

Both silymarin and honey, alone or mixed with black seed, administration led to a significant increase in serum total protein and albumin and a significant decrease in serum total cholesterol and triacylglycerols levels when compared with group II. These results coincide with Freitag *et al*. [61], Wang *et al*. [62], and Saricicek *et al*. [69]. This can be related to the antioxidant properties of silymarin, honey, and black seed, which shelter hepatocytes from $TASO_2$-induced oxidative damage. This ensured the functionality of a larger number of hepatocytes than in case of group II. However, serum total protein and albumin levels of groups IV, V, and VI increased significantly in comparison with control group in coincidence with Amin *et al*. [66] and Saricicek *et al*. [69]. This may be because honey and black seed enhance healing and regenerating of injured hepatocytes through increasing the rate of protein synthesis.

A significant declination in fasting plasma glucose level from normal range occurs only in cases of hepatic failure owing to severe damage as in the last stage of cirrhosis [66]. This late stage was not reached in our study, so fasting blood glucose level remained in the normal range in all the experimental groups.

TA administration resulted in a great damage to rats' liver. This was obvious in the nodular appearance of the liver lobule, bridging necrosis, and vacuolar degeneration of hepatocytes. These results are in accordance with De David *et al*. [72] and coincide with Chen *et al*. [68]. This also agrees with our biochemical analyses results. TA causes centrilobular necrosis, which stimulates the replacement of parenchymal cells by permanent ECM, which forms scars [2].

Treatment with silymarin and honey alone or mixed with black seed led to an improvement in liver architecture. This was shown in the reduced fibrotic areas and less inflammation in comparison with group II. These results agree with Freitag *et al*. [61], Wang *et al*. [62], and Saricicek *et al*. [69] and are in accordance with our biochemical analyses results.

The correlation found between Gal-3 and TGF-β1 may be owing to the direct effect of Gal-3 on activation of TGF-β1 expression. Gal-3 stimulates macrophages to secrete TNF-α, which triggers activation of the transcriptional factor NF-κB [45,50], which in turn triggers TGF-β1expression. We found that Gal-3 positively correlates with fibrotic area, which agrees with Li *et al.* [46]. Regarding the correlation between liver Gal-3 and each of liver TAC, MDA, NO levels, catalase and serum ALT and AST activities, this may be owing to the induced oxidative stress, which is the common factor resulted in the elevation of all these parameters levels. Both honey and black seed are natural products with no adverse effects and no limitations when used at suitable doses. The correlated equivalent human dose for the mixture (100 mg/kg) can be calculated according to Jang-Woo *et al.* [73], and it is 16 mg/kg b. wt per day.

Conclusion

In conclusion, the results of this study showed an inhibitory effect of honey alone or mixed with black seed on Gal-3 level in rats' liver after TA-induced injury. The mixture at dose 100 mg/kg had greater effect on inhibiting Gal-3 level and improving liver state than using honey alone and was closer to silymarin effect. Moreover, the mixture at dose 100 mg/kg had greater ability in fighting oxidative stress than both

mixture dose 50 mg/kg and honey. This inhibitory effect on Gal-3 can be related to the direct effect of honey and black seed on Gal-3 expression through inhibiting the transcriptional factors NF-κB and AP-1, along with an indirect role of honey and black seed in protecting against TA-induced oxidative stress. Gal-3 had significant positive correlations with other liver disease biomarkers, which introduce it to be a significant indicator for screening liver disease progression.

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Conflicts of interest
There are no conflicts of interest.

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