The Protective Effect of Quercetin on Thioacetamide- Induced Liver Cirrhosis in Adult Male Albino Rats

Original Article

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ABSTRACT

Introduction: Liver Cirrhosis is a major health problem affecting many people worldwide. To date, no efficient treatment approach has developed for this disease.

Objectives: The current study was conducted to evaluate the potential of quercetin (QR) to protect the liver from thioacetamide (TAA)-induced fibrosis in rats using histological, histochemical, biochemical and morphometric studies.

Material and Methods: The study included forty adult male albino mice. Four groups of male rats were treated as follows: group 1 was the control group, group 2 was given QR (50 mg/kg/day) orally, group 3 was administered TAA (200 mg/kg i.p), twice weekly, and group 4 was given TAA (200 mg/kg i.p) twice weekly and QR (50 mg/kg/day) orally. Animal treatment was continued for eight weeks. After 8 weeks, all rats were weighed then sacrificed; blood samples were taken for determination of serum alanine aminotransferase (ALT), and alkaline phosphatase (AP), livers were removed, photographed, and used for histopathological examination by H&E and Sirius red stains.

Results: QR administration protected against TAA hepatotoxicity, as evidenced by increased weight gain , increased liver heaviness and significant inhibition of serum ALT and AP activity rise caused by TAA. Gross examination of TAA rats showed liver cirrhosis with variable size nodules that were reduced in TAA/QR livers. Histopathological examination of rat livers revealed a loss of normal liver architecture (very thick septa and inflammatory infiltration). TAA/QR rat livers, on the other hand, had almost normal hepatic architecture.

Conclusion: The natural flavonoid QR could ameliorate TAA-induced liver cirrhosis and liver dysfunction in adult male albino rats.

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Key Words: Liver cirrhosis; quercetin; serum liver enzymes; thioacetamide.

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INTRODUCTION

Cirrhosis, the last stage of liver fibrosis, is one of the world's most serious health problems. It is linked to an increased risk of morbidity and mortality, which has serious health and social implications. It is ranked 13th overall and fourth among chronic diseases according to mortality.[1] It is defined by the formation of excess extracellular matrix and collagen in reaction to persistent liver cell injury, and it is the final step in the progression of many chronic liver disorders.[1]

Fibrosis and cirrhosis are long-term wound healing outcomes to chronic liver injury caused by infectious, autoimmune, drug-induced, cholestatic, and metabolic diseases.[2]

The hepatotoxin thioacetamide (TAA) is a very efficient, dependable, and satisfying model for developing liver cirrhosis in laboratory animals.[3]

TAA is employed at varying intervals to promote liver cirrhosis, severe bile duct growth, and cholangiocarcinoma. Liver damage is correlated with many biochemical changes as elevated levels of serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP).[4]

Histopathological changes include vascular congestion and inflammatory infiltration with congestion of sinusoids, centrilobular necrosis, as well as nuclear changes.[4]

Oxidative stress, hepatic tissue inflammation, stellate cell activation, and fibrosis are all factors in the progression of hepatic cirrhosis.[5]

In response to hepatic damage, the autocrine and paracrine release of fibrogenic cytokines accelerates HSC transformation from a dormant (quiescent) state to an active myofibroblastic state with migratory and rapidly proliferative characteristics.

TGF- β is one of the well-known cytokines involved in fibrogenesis pathways. Other cytokines include tumor necrosis factor (TNF-α), interleukin 1 (IL-1), and platelet-derived growth factor (PDGF).[6]

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TGF-β1 is regarded as the maestro fibrogenic cytokine, with a long history of playing a key role in liver fibrosis in a variety of illnesses. TGF1 regulates key fibrogenic events in myofibroblasts (MFBs) and other cells through its receptors, including MFB pool expansion, extracellular matrix protein activation, and matrix metalloproteinase inhibition.[7, 8]

Flavonoids are phenolic phytochemical molecules that can be found in high amounts in a variety of foods, such as fruits, vegetables, seeds, and beverages like green tea and wine. Because of their antioxidant features and ability to protect cells from reactive oxygen species, flavonoids are considered semi-essential nutrients for humans.[5]

Antioxidants, such as flavonoids, have also been widely researched for acute and chronic liver disease prevention and treatment, with encouraging results.[9,10]

In this context, quercetin (QR) is one of the most studied flavonoids, with excellent free radical scavenging properties.[5] It is present in large amounts in apples, berries, onions, citrus fruits, cruciferous vegetables, tea, pepper, tomato, whole grain, cocoa, and red wine having a wide range of health benefits. It contains several phenolic hydroxyl groups and is a powerful oxygen-free radical scavenger and a metal chelating agent.[11]

QR has potent antioxidant and cytoprotective properties,[12] as well as anti-inflammatory, hepato-protective, reno-protective, and neuro-protective properties.[13,14] Quercetin appears to have the ability to prevent experimental liver cirrhosis, according to some evidence.[15,16]

This study aimed to estimate the hepatoprotective effects of the flavonoid QR against TAA-triggered liver cirrhosis in male rats using histological, histochemical, biochemical and morphometric studies.

MATERIAL AND METHODS

Test Compounds: (chemicals)

a. Thioacetamide (TAA) was obtained from Sigma Chemical, Co., Ltd. (St. Louis, MO, United States) and dissolved in normal saline for intraperitoneal injection. TAA was prepared freshly by dissolving 200mg/kg body weight in 1ml sodium chloride 0.9%. Then, the solution was administered intraperitoneally (ip) to the rats two times weekly for 8 weeks.

b. Quercetin was obtained from Sigma Chemical, Co., Ltd. (St. Louis, MO, United States) and dissolved in sterile distilled water and given to rats in dosages of 50 mg/kg body weight daily via orogastric tube for 8 weeks.

The equivalent human dose of QR used in this study is 10–30 times higher than the average human daily intake on a weight-to-weight basis.[17]

Experimental animals

- The research was conducted out on forty male Sprague-Dawley rats (weigh approximately 200-250 g) acquired from the Faculty of Medicine's Animal House Center of Anatomy Department.
- Rats were housed two per cage at room temperature maintained at twenty degrees (20◦C) on a 12 hour light and 12 hour dark cycle. Diets were administrated following the Egyptian Institute of Nutrition (EIN) recommendations. During the experiment, the animals were allowed to eat and drink without being restrained.

Experimental design

- Rats were divided into four equal groups at random
  1. **Group I:** Control group (n= 10); C rats were given sodium chloride 0.9% (1mg/kg, single dose) by intraperitoneal injection twice weekly for eight weeks.
  2. **Group II:** Quercetin group (n= 10); QR rats were treated with Quercetin (50 mg/kg/day) orally for eight weeks.
  3. **Group III:** Thioacetamide group (n= 10); TAA rats were treated with thioacetamide in a dose of 200 mg/kg, by intraperitoneal injection twice weekly for eight weeks.[18]
  4. **Group IV:** Thioacetamide & Quercetin group(n= 10); TAA/QR rats were treated with thioacetamide in a dose of 200 mg/kg, by intraperitoneal injection twice weekly and Quercetin (50 mg/kg/day) orally for eight weeks.[18]
- Animals' weight was measured before and after the experiment to measure the weight gain.
- At the end of the treatment period, under light ketamine/xylazine anesthesia, all animals were sacrificed. A cardiac puncture was used to draw blood, and the liver was quickly removed for examination.
- Dissected rat livers were washed in normal saline, blotted using filter sheets, weighed, and inspected for any gross changes.
- The following formula was used to determine the liver index: (liver wet weight/body weight) x100 = liver index.[19]

Serum enzyme activities

To obtain the serum, blood samples were centrifuged at 1200 g for 15 minutes. The activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were then measured to determine liver damage (AP).

Gross Macroscopic study

Livers were examined and photographed at the experimental embryology lab, Anatomy and Embryology Department, Faculty of Medicine, Alexandria University, using Olympus SZ dissecting stereomicroscope. Any gross anatomical changes were recorded.

Histopathological examinations

Liver samples were obtained from all the animals and fixed with 10% formal saline and processed to get five-micrometer thick paraffin sections. These sections were stained for light microscopic examination.[18] Paraffin sections were stained by Haematoxylin and Eosin (H&E) to evaluate liver architectural changes and Sirius Red stain (a special stain for collagen fibers) to determine the degree of fibrosis.[20,21]

Morphometric analysis of staining surface area of Sirius Red in addition to histopathological scores were also done. Slides were viewed and photographed using a Leica microscope in the Pathology Department, Alexandria University.

Morphometric analysis for collagen fibers deposition in Sirius Red stained liver sections:

The Ishak *et al* scoring method was chosen to quantify the degree of fibrosis since it is favored for research, reproducible, and validated in clinical research. "0 = fibrosis not present, 1 = small portal area expansion with/without septa, 2 = fibrous expansion of most portal areas with/without septa, 3 = most portal area fibrous expansion fibrous with infrequent portal to portal bridging, 4 =fibrous dilatation of most portal areas with portal to portal and portal to central bridging, 5 = signified bridging with formation of nodules (partial cirrhosis), 6 = cirrhosis evident or definite.[22,23]

Digital images were acquired from Sirius red-stained sections using a digital camera linked to the microscope at magnification x400 (n=4/group). Measurements were represented as a percentage fibrotic area to the total surface area using Leica Image analysis software. The Ishaq scoring system was used to stage the animals based on the percentage of fibrotic surface area.[22,23]

Immunohistochemical study

Tissue sections 3–5 µm thick sections were obtained and placed on positively charged slides. The immunostaining procedure was done according to the manufacture's protocol. The primary antibodies transforming growth factor β1 (TGFβ1) were diluted (at 1:800) and incubated for 20 minutes at room temperature. All of the reactions were conducted with the help of appropriate positive and negative controls. Finally, the slides were counterstained with haematoxylin.[24]

Image software was used to determine the intensity of TGFβ1 immunostaining. A colour histogram was created from five random portal and lobular patches from each slide using a colour deconvolution plug-in commonly used for diaminobenzidine staining.

Statistical analysis

The IBM SPSS software program version 20.0 was used to examine the data supplied into the computer. The data were given in the form of a mean and a standard deviation (SD).[25]

The student "t" test was used to compare the differences between two continuous variables. Using Scheffe's approach for multiple comparisons, differences between three or more continuous variables were analyzed using a one-way analysis of variance (ANOVA). The Chi-squared and Fisher's exact tests were used to compare proportional differences. The significance level was set at $p \leq 0.05$.[25]

RESULTS

Weight gain

In the current work, the mean weight gain in group I (Control group) was 98.10 ± 15.61 gm. In group II (QR group), the mean weight gain value was as nearly as that of the control group, with no significant variation.

There was a significant reduction in body weight gain in TAA group compared to the control group (P<0.001). The mean body weight gain in group III was 29.0 ± 2.98gm.

However, the body weight gain in TAA/QR group was significantly increased (P4<0.001) compared to group III; its mean body weight gain was 59.70 ± 9.86 gm. (Table 1)

Liver weight

Mean liver weight in the control group was 8.53 ± 0.60 gm. In the QR group, it was 8.65 ± 0.67gm. There wasn't any significant difference in comparison to the control group. Mean liver weight in group III was significantly increased to 11.44 ± 0.87gm (P<0.001). In group IV, the mean liver weight was 9.02 ± 1.04 gm. So, there was a significant reduction in liver weight compared to group III (P4<0.001). (Table 2)

Liver index (liver weight / body weight X100)

Mean liver index in control group (group I) and QR group (group II) was 2.68 ± 0.29 and 2.70 ± 0.20 respectively, so there wasn't any significant difference between both groups.

Mean liver index in TAA group (group III) increased to 4.59 ± 0.39. This increase was statistically significant (p<0.001). This means increase in liver heaviness in relation to the body.

In rats treated with QR in addition to TAA (TAA/QR group), mean liver index was reduced to 3.19 ± 0.42. this reduction was statistically significant (p4<0.001) as compared to hepatotoxic group (group III). (Table 2)

Biochemical results

Serum ALT

In the present work, the mean level of serum ALT in group I (control group), was 29.20 ± 8.40. In group II (QR group), the mean value was 34.30 ± 3.65 this was as nearly as that of the control group, with no significant difference. Following the administration of TAA, the mean level of ALT in group III was increased significantly (P<0.001) as compared to the control group, the mean value of ALT in this group was 290.9 ± 29.17. In contrast to group IV (TAA/QR group), serum ALT level was decreased significantly (P4<0.001) as compared to the TAA group showing a mean of 48.20 ± 4.26. (Table 3)

Serum Alkaline Phosphatase (AP)

The mean level of serum AP in group II (QR group), was 199.4 ± 18.03 which was non-significant as compared with group I (Control group) in which the mean AP level was 191.6 ±26.0, while in group III (TAA group), the mean value significantly increased to 514.9 ± 86.66.

However, serum AP mean value in group IV (TAA/QR group) was reduced significantly as compared to group III (TAA group) showing a level of 292.9 ± 17.21.(Table 3)

Liver anatomical gross feature results

Gross macroscopic examination of control rats' livers revealed normal anatomical appearance with smooth red glistening surfaces. QR group rat livers also showed preserved normal liver gross picture with smooth glistening surfaces.

In group III (TAA group), TAA exposure caused the liver to take an irregular shape. It was also occupied by micronodules and macronodules (whitish granules), some of which were close to the surface. Cirrhosis in humans is characterised by nodular change of the liver parenchyma.

In group IV rats, therapy with QR prevented the formation of macro-and micronodules and let the liver maintain its nearly normal anatomical shape and appearance. Even though these findings were based on visual inspections, they clearly revealed that the QR successfully prevented the formation of nodules and thereby preserved the liver from further structural and functional degeneration. (Figure 1)

Histopathological Examination

Hematoxylin and Eosin results

In Control group, there was preserved liver architecture. Portal tracts and central veins were in normal size. In QR group, liver architecture was also preserved, portal tracts were normal in size, but central veins were slightly dilated. However, TAA group showed loss of normal hepatic architecture as it was replaced by regenerative nodules of variable sizes surrounded by thick fibrosis and inflammatory infiltrate (liver cirrhosis). TAA /QR rats' livers revealed preserved hepatic architecture. The hepatic tissue was characterized by focal appearance of fibrous tissue bands, broadening of portal tracts and congested central veins surrounded with minimal amount of cellular infiltrations. (Figure 2)

Sirius Red results

The examination of sirius red stained liver tissue was performed to determine the degree of fibrosis and collagen deposition. Collagen fibres attained the red colour of Sirius red, while the remaining hepatic tissue appeared with yellow colour.

Control group rat liver examination revealed normal yellow hepatic tissue with minimal collagen deposition surrounding central veins and portal tracts and in the liver capsule. Also, examination of group II livers showed also normal hepatic tissue with few collagen fibers in the capsule of the liver and around the central veins and portal tracts.

TAA administration resulted in a progressive increase in collagen fiber deposition in the liver tissue. Collagen deposition was in the form of numerous periportal fibrous connective tissue septa. These septa formed bridges connecting adjacent portal areas resulting in pseudo-lobulation and appearance regenerative nodules of different sizes (macro and micronodules) which is a main feature of liver cirrhosis.

Rat liver sections of subgroup IV showed nearly normal distribution of collagen around the central veins and portal tracts with focal appearance of small collagen bands. that also represents evidence for QR's protective effect against collagen fiber deposition and progression to liver cirrhosis. (Figure 3)

Morphometric analysis for collagen fibers deposition in Sirius Red stained liver sections

Sirius red-stained specimens were also examined by a blind pathologist to assess the portal tract expansion, presence of fibrous septa and their extent, bridging of septa and the formation of regenerative nodules according to the Ishaq scoring system (scores from 0-6).

Both control and QR groups revealed normal hepatic tissue with no fibrosis (Score 0), while TAA group showed marked portal tract expansion with the appearance of marked bridging fibrous septa and the presence of nodules of variable sizes (Score 5).

TAA/QR protected group depicted a marked statistically significant decrease in fibrosis with few focal fibrous septa, minor broadening of portal tracts and preservation of normal hepatic architecture (Score 1). (Table 4)

Digital images were taken from Sirius red-stained sections using a digital camera linked to a Leica microscope at magnification x 400 (n=10/group). Using Leica Image software, measurements were reported as a percentage of surface area.

The mean area percentage of collagen fibers did not exhibit any significant difference in control and QR groups. It was significantly increased in TAA group ($p \leq 0.001$) with respect to the control. Meanwhile, it was lowered in TAA/QR treated groups as compared to TAA group, but the difference was non-significant ($p4 = 0.056$). (Table 4)

Immunohistochemistry results

TGF-β expression immunohistochemistry of the control group revealed mildly immunoreacted TGF-βpositive cells around central veins and blood vessels of portal tracts whereas other liver cells were negative. Immunohistochemical evaluation of TGF-β expression in QR group showed nearly the same picture of control group with non-specific TGF-β expression of hepatic tissue.

While immunohistochemical evaluation of TGF-βexpression of group III revealed a marked increase of immunoreacted TGF-β b positive fibroblast cells detected around the central veins and vessels of portal tracts. Immunopositively reacted cells were also detected in between hepatocytes and forming bridges with septa formation. On high power magnification, these cells appeared spindle shaped, with many processes.

QR/TAA revealed a staining pattern nearly like the control groups. Mildly immunoreacted TGF-β positive cells surrounding central veins and portal tracts were observed. A few positive immunoreacted fibroblast cells were observed forming few small bridges. (Figure 4)

Morphometric analysis for immunohistochemical evaluation of TGF-β expression

Control and QR groups showed no significant variance in the expression of immunoreacted TGF-β positive cells. The TAA group showed a significant rise ($p \leq 0.001$) in TGF-β immunopositivity in comparison to the control groups. The TGF-β immunopositive cells were significantly reduced in the TAA/QR group when compared to the TAA group ($p \leq 0.001$) as shown in (Table 5).

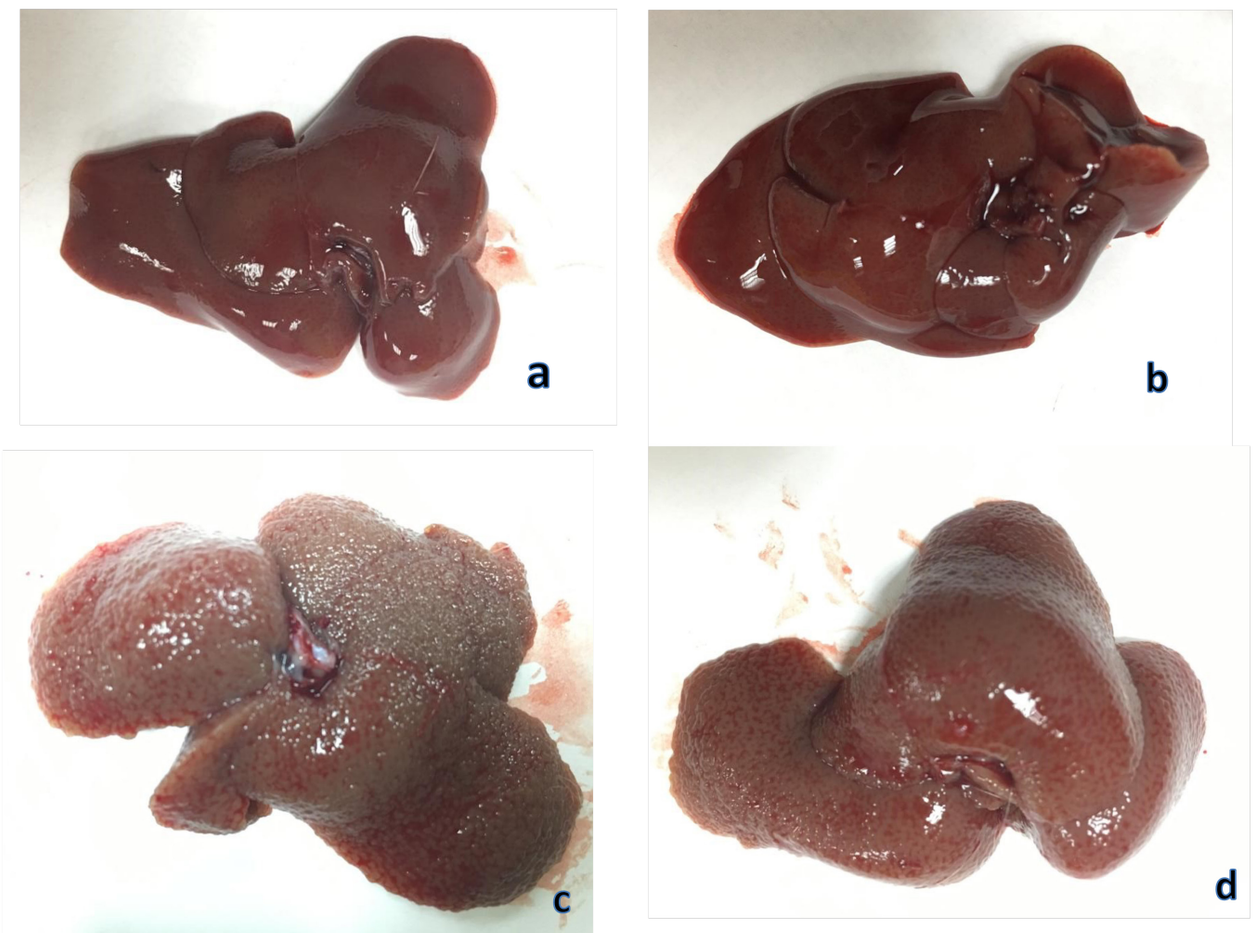

Fig. 1: a) Macroscopic image showing the macroscopic appearance of liver of group I (Control) showing regular smooth glistening appearance. **b)** Macroscopic image showing the macroscopic appearance of liver of group II (QR group) showing preserved normal regular smooth glistening appearance. **c)** Macroscopic image showing the macroscopic appearance of liver of group III (TAA group) showing loss of normal hepatic appearance with irregular whitish micro- and macronodules. **d)** Macroscopic image showing the macroscopic appearance of liver of group IV (TAA/QR group) preserved nearly normal liver appearance with markedly less appearance of macro- and micronodules.

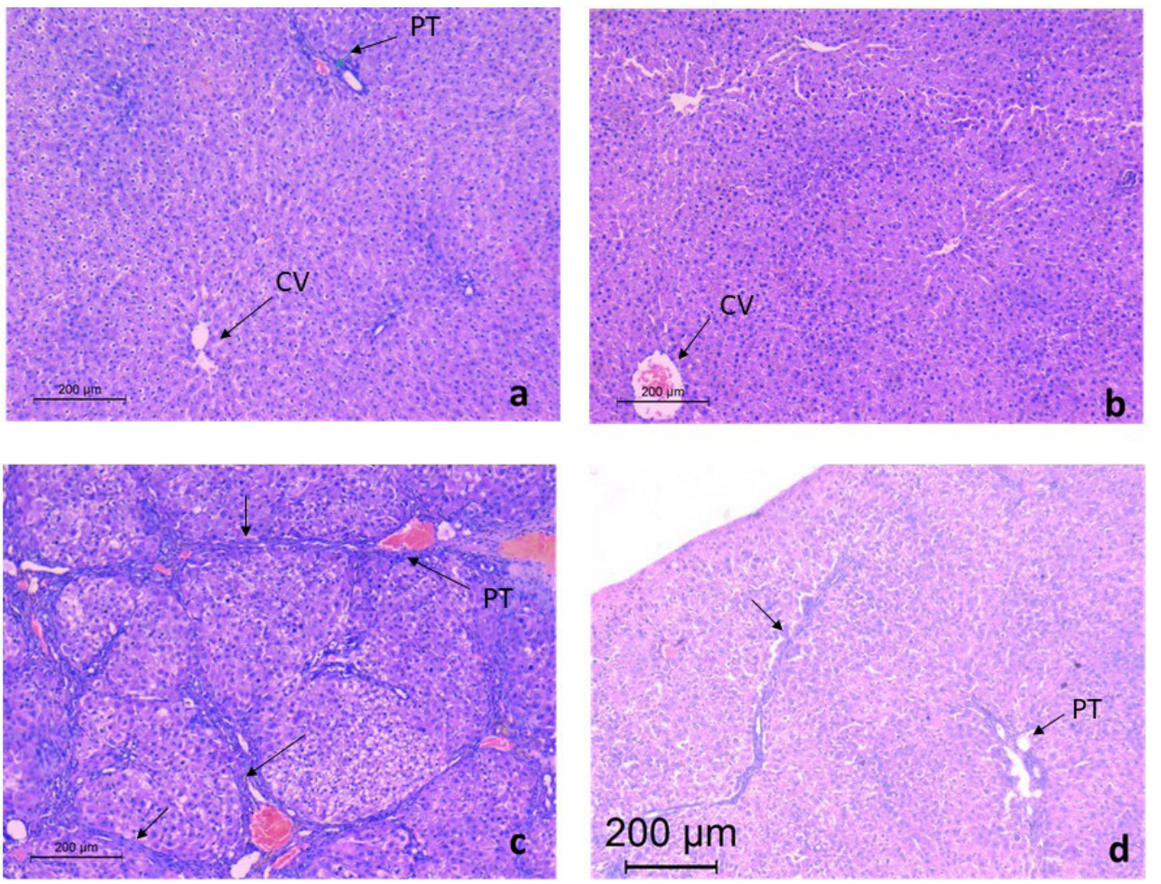

Fig. 2: a) A photomicrograph of section of liver of control group showing preserved hepatic architecture. (H & E, 100 X). **b)** A photomicrograph of section of liver of QR group also showing normal hepatic lobule with congested central vein (H & E, 100 X). **c)** A photomicrograph of section of liver of TAA group showing lost hepatic architecture replaced by nodules of variable sizes separated by very thick fibrous septa (black arrows) around congested central veins, cellular infiltration and congested blood sinusoid (H & E, 100 X). **d)** A photomicrograph of section of liver of TAA / QR group showing preserved architecture with broadening of portal tracts and few fibrous bands (black arrow) (H & E, 100 X). CV: central vein, PT: portal tract.

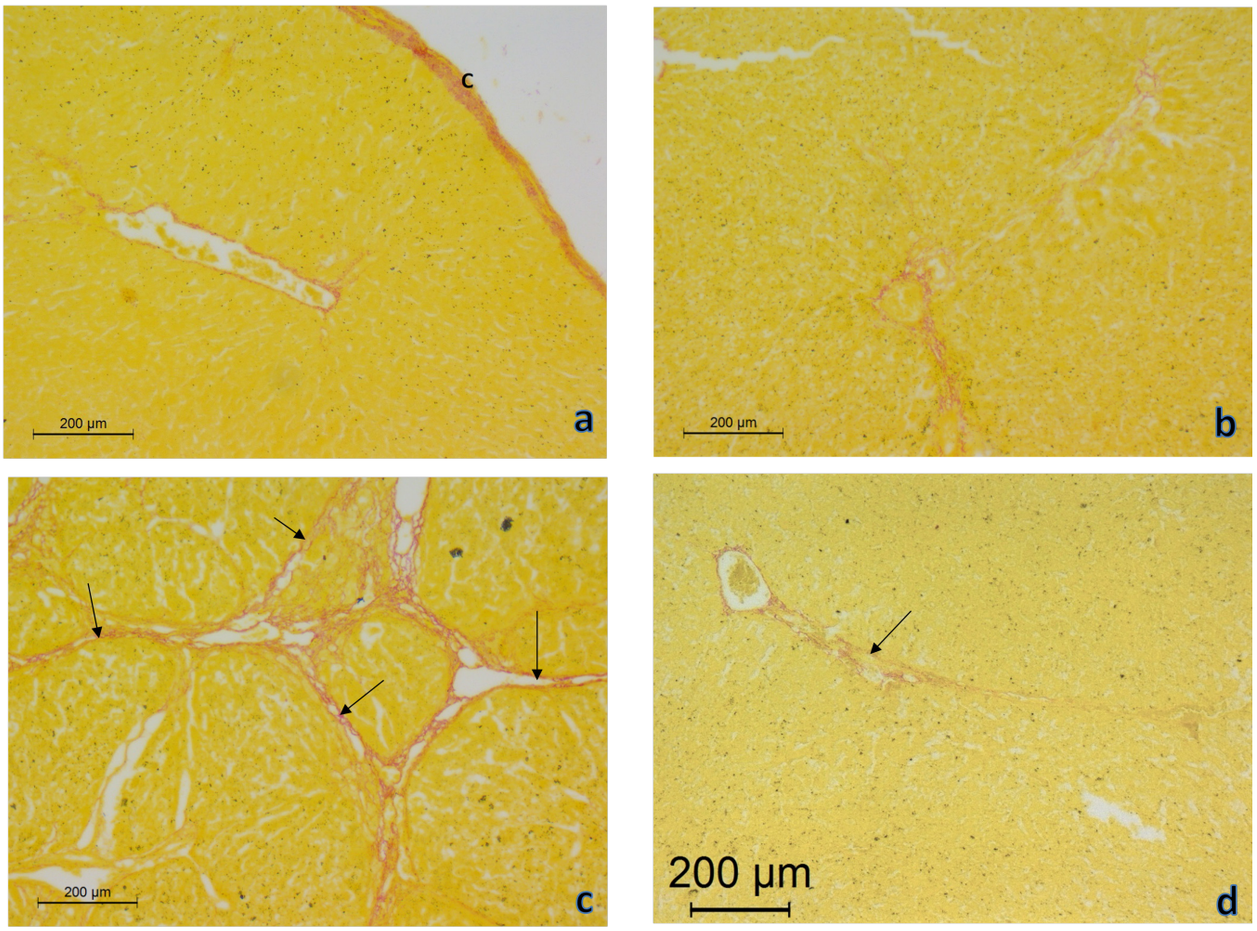

Fig. 3: a) A photomicrograph of section of liver of group I (Control group) showing normal hepatic tissue stained yellow with no red stained collagen except at the fibrous capsule (C) (Sirius red, 100X) **b)** A photomicrograph of section of liver of group II (QR group) showing preserved hepatic tissue stained yellow with no red stained collagen (Sirius red, 100X). **c)** A photomicrograph of section of liver of group III (TAA group) showing distorted hepatic architecture in the form of hepatic nodules stained by yellow color separated by bands of bridging fibrosis stained by red color (arrows) (Sirius red, 100X). **d)** A photomicrograph of section of liver of group IV (TAA/QR group) showing preserved hepatic architecture with focal red stained fibrous tissue bands stained by red color (arrow) (Sirius red, 100X).

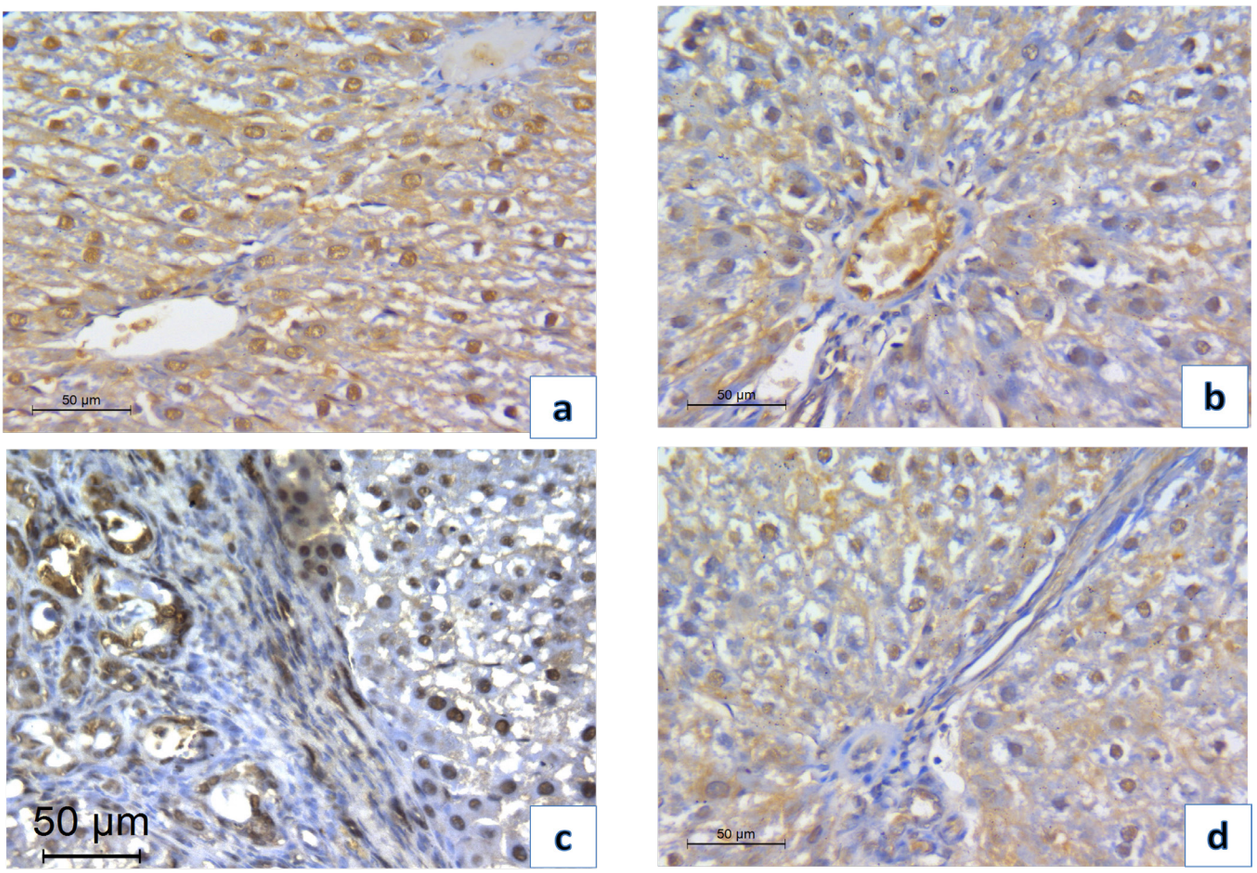

Fig. 4: **a)** A photomicrograph of control rat liver Group I for immunohistochemical evaluation of TGF-β expression showing negatively immunoreacted cells (TGF-β counterstained with haematoxylin, X400). **b)** A photomicrograph of rat liver Group II (QR group) for immunohistochemical evaluation of TGF-β expression showing no positively stained cells (TGF-β counterstained with haematoxylin, X400). **c)** A photomicrograph of rat liver Group III (TAA group)for immunohistochemical evaluation of TGF-β expression showing numerous strong immunoreacted cytoplasmic staining in spindle cells in fibrous bands between cirrhotic nodules (TGF-β counterstained with haematoxylin, X400). **d)** A photomicrographs of rat liver Group IV (TAA/QR group)for immunohistochemical evaluation of TGF-β expression showing mild immunoreacted cytoplasmic staining in spindle cells (TGF-β counterstained with haematoxylin, X400).

Table 1: Comparison between the different studied groups according to rat weight gain.

	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	F	p
Weight gain						
Min. – Max.	74.0 – 128.0	80.0 – 112.0	25.0 – 34.0	39.0 – 71.0		
Mean ± SD.	98.10 ± 15.61	97.50 ± 10.47	29.0 ± 2.98	59.70 ± 9.86	96.625*	<0.001*
Median (IQR)	98.0 (87.0 – 108.0)	99.50 (93.0 – 103.0)	28.50 (27.0 – 31.0)	61.50 (54.0 – 67.0)		
p_1		0.999	<0.001*	<0.001*		
Sig. bet. grps.			p_2<0.001*,p_3<0.001*,p_4<0.001*			

SD: Standard deviation IQR: Inter quartile range
F: F for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups
p_1: *p value* for comparing between Group I and each other groups
p_2: *p value* for comparing between Group II and Group III
p_3: *p value* for comparing between Group II and Group IV
p_4: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$

Table 2: Comparison between the different studied groups according to liver weight and liver index

	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	F	*P*
Liver weight						
Min. – Max.	7.43 – 9.54	7.80 – 9.90	10.20 – 12.80	6.90 – 9.90		
Mean ± SD.	8.53 ± 0.60	8.65 ± 0.67	11.44 ± 0.87	9.02 ± 1.04	96.625*	<0.001*
Median (IQR)	8.54 (8.2 – 8.9)	8.55 (8.1 – 9.1)	11.65 (10.5 – 11.9)	9.35 (8.5 – 9.9)		
p_1		0.989	<0.001*	0.544		
Sig. bet. grps.			p_2<0.001*,p_3=0.737,p_4<0.001*			
Liver index						
Min. – Max.	2.20 – 3.16	2.40 – 2.96	4.02 – 5.19	2.50 – 3.84		
Mean ± SD.	2.68 ± 0.29	2.70 ± 0.20	4.59 ± 0.39	3.19 ± 0.42	28.488*	<0.001*
Median (IQR)	2.66 (2.5 – 2.9)	2.69 (2.6 – 2.9)	4.53 (4.3 – 4.9)	3.19 (2.8 – 3.5)		
p_1		0.999	<0.001*	0.010*		
Sig. bet. grps.			p_2<0.001*, p_3=0.013*,p_4<0.001*			

SD: Standard deviation
p: *p value* for comparing between the studied groups
p1: *p value* for comparing between Group I and each other groups
p2: *p value* for comparing between Group II and Group III
p3: *p value* for comparing between Group II and Group IV
p_4: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$

Table 3: Comparison between the different studied groups according to laboratory investigations

	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	Test of Sig.	p
Alanine aminotransferase (ALT)						
Min. – Max.	15.0 – 39.0	29.0 – 39.0	230.0 – 321.0	41.0 – 55.0		
Mean ± SD.	29.20 ± 8.40	34.30 ± 3.65	290.9 ± 29.17	48.20 ± 4.26	F= 677.971*	<0.001*
Median (IQR)	31.50 (25.0 – 34.0)	34.50 (31.0 – 38.0)	295.5 (284.0–316.0)	48.50 (46.0 – 51.0)		
p_1		0.881	<0.001*	0.044*		
Sig. bet. grps.			p_2<0.001*,p_3=0.202,p_4<0.001*			
Alkaline phosphatase (AP)						
Min. – Max.	142.0 – 213.0	165.0 – 222.0	412.0 – 618.0	258.0 – 321.0		
Mean ± SD.	191.9 ± 26.0	199.4 ± 18.03	514.9 ± 86.66	292.9 ± 17.21	F= 103.009*	<0.001*
Median (IQR)	201.0 (189.0 – 210.0)	203.0 (189.0 – 211.0)	529.0 (430.0 – 598.0)	292.5 (287.0 – 302.0)		
p_1		0.984	<0.001*	<0.001*		
Sig. bet. grps.			p_2<0.001*,p_3<0.001*,p_4<0.001*			

SD: Standard deviation IQR: Inter quartile range
F: F for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
p: *p value* for comparing between the studied groups
p_1: *p value* for comparing between Group I and each other groups
p_2: *p value* for comparing between Group II and Group III
p_3: *p value* for comparing between Group II and Group IV
p_4: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$

Table 4: Comparison between the different studied groups according to grade of fibrosis and collagen surface area percent

	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	H	*p*
Grade of fibrosis						
0	10 (100.0%)	10 (100.0%)	0 (0.0%)	0 (0.0%)		
I	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (70.0%)		
II	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (30.0%)	38.024*	<0.001*
III	0 (0.0%)	0 (0.0%)	6 (60.0)	0 (0.0%)		
IV	0 (0.0%)	0 (0.0%)	3 (30.0%)	0 (0.0%)		
V	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)		
p_1		1.000	<0.001*	0.002*		
Sig. bet. grps.			p_2<0.001*,p_3=0.002*,p_4=0.040*			
Collagen surface area percent						
Min. – Max.	0.003 – 0.054	0.012 – 0.080	5.34 – 22.87	1.09 – 5.32		
Mean ± SD.	0.25 ± 0.015	0.030 ± 0.021	9.97 ± 5.53	2.69 ± 1.41	32.933*	<0.001*
Median (IQR)	0.025 (0.01 – 0.03)	0.021 (0.02 – 0.04)	7.37 (6.2 – 12.4)	2.34 (1.7 – 3.2)		
p_1		0.939	<0.001*	0.004*		
Sig. bet. grps.			p_2<0.001*,p_3=0.005*,p_4=0.056			

SD: Standard deviation IQR: Inter quartile range
H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
p: *p value* for comparing between the studied groups
p_1: *p value* for comparing between Group I and each other groups
p_2: *p value* for comparing between Group II and Group III
p_3: *p value* for comparing between Group II and Group IV
p_4: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$

Table 5: Comparison between the different studied groups according to immune score

Immune score	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)
0	10 (100.0%)	10 (100.0%)	0 (0.0%)	0 (0.0%)
1	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (60.0%)
2	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (40.0%)
3	0 (0.0%)	0 (0.0%)	2 (20.0%)	0 (0.0%)
4	0 (0.0%)	0 (0.0%)	5 (50.0%)	0 (0.0%)
5	0 (0.0%)	0 (0.0%)	3 (30.0%)	0 (0.0%)
Min. – Max.	0.0 – 0.0	0.0 – 0.0	3.0 – 5.0	1.0 – 2.0
Mean ± SD.	0.0 ± 0.0	0.0 ± 0.0	4.10 ± 0.74	1.40 ± 0.52
Median (IQR)	0.0 (–)	0.0 (–)	4.0 (4.0 – 5.0)	1.0 (1.0 – 2.0)
H (p)		37.905* (<0.001*)		
p_1		1.000	<0.001*	0.002*
Sig. bet. grps.			p_2<0.001*, p_3=0.002*,p_4=0.040*	

SD: Standard deviation IQR: Inter quartile range
H: H for Kruskal Wallis test, pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test)
p: *p value* for comparing between the studied groups
p_1: *p value* for comparing between Group I and each other groups
p_2: *p value* for comparing between Group II and Group III
p_3: *p value* for comparing between Group II and Group IV
p_4: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$

DISCUSSION

The present study evaluates the QR's ability to protect against TAA-induced liver cirrhosis. TAA is a hepatotoxin that is used for experimental induction of hepatic fibrosis and cirrhosis. TAA metabolism releases thioacetamide-S dioxide, a highly reactive chemical that binds to tissue macromolecules and causes severe liver damage.[26]

In the current study, rats subjected to thioacetamide-induced liver toxicity showed a significant decrease in weight gain that was possibly correlated to decreased appetite caused by altered brain serotonin (5-HT) metabolism and elevated brain 5-HT levels which plays an important role in the pathogenesis of anorexia linked to TAA-induced liver cirrhosis according to Haider *et al*.[27] This decrease in weight gain caused by TAA was significantly corrected in TAA/QR group. Hamed *et al*.,[4] also reported increase in final body weight and BMI of rats protected by QR intake which agree with the present study.

In the present study, liver weight and liver index were significantly increased in TAA rats. Abood *et al*.,[28] also depicted increase in both liver weight and liver index after chronic TAA injection (200 mg/kg) three times per week for 8 weeks in their study that was conducted over 36 rats to evaluate the protective effects of garcinia mangostana peels extract on TAA induced liver cirrhosis.

The biochemical study revealed hepatic damage in the form of raised serum levels of liver enzymes, this picture described by numerous authors with multiple hepatotoxic agents that cause liver fibrosis or cirrhosis.[4,9] Thioacetamide causes liver damage by interfering with protein synthesis and enzyme metabolism, according to Liu *et al*.[29]

In the present study, gross inspection of TAA-treated group rats revealed liver cirrhosis. The surface of the liver was rough and nodular, with homogeneous micro-nodules and macronodules on the surface. Abood *et al*.,[28] also depicted the presence of variable-sized nodules disrupting the surface of the liver on gross examination after 8-week treatment with ip TAA (200mg/kg body weight) three times weekly.

Histological examination of liver of TAA group by H&E revealed lost normal hepatic architecture that has been replaced by regenerative nodules of variable sizes surrounded by thick fibrosis and inflammatory infiltrate (liver cirrhosis). These findings are consistent with those of Hamed *et al*.,[4] who found that hepatic tissue architecture was disrupted by the presence of very thick fibrous septa containing many fibroblasts between hepatic lobules, around central veins, and portal tracts.

The collagen distribution in the liver was detected and the amount of hepatic fibrosis was estimated using Sirius red staining. TAA-treated rats' livers displayed evident collagen accumulation between liver lobules, which is a common characteristic of hepatic cirrhosis. Collagen surface area was measured by image analysis and revealed statistically significant rise in TAA hepatotoxic group. Based on Sirius red staining and morphometry, Traber *et al*.,[30] observed significant fibrosis compared to the entire area of the liver.

In the present experiment, TGFβ1 immunohistochemistry was used to assess the intensity of TGFβ1 in the liver. Livers of the TAA group showed a statistically significant increase in immune expression. This result was also depicted by Abood *et al*.[28]

Vegetables, Fruits, tea, and spices provide essential nutrients as well as a variety of diet-derived phenolics, particularly flavonoids, which have been linked to anticarcinogenic activity.[5,9,10] One of the most frequent flavonoids found in the diet is QR.[5]

In the present study, rats treated with QR orally at the same treatment period with hepatotoxic compound TAA showed signs of improvement by biochemical and histological studies. This may be explained by the ability of QR to potentiate the antioxidant defense in the liver thus breaking the vicious cycle of oxidative stress and liver damage. Afifi *et al*.,[12] stated that this improvement in the liver functions have been shown by the significant decrease of the abnormally high liver enzymes.

The decrease in weight gain caused by TAA was significantly corrected in TAA/QR group. Hamed *et al*.,[4] also reported increase in final body weight and BMI of rats protected by QR intake which agree with the present study.

When compared to the TAA group, QR-fed rats had lower liver weights that were close to normal. The drop in liver weight and index seen in QR-fed rats could be attributed to a reduction in hyperlipidemia or to a decrease in inflammation.[31]

In the present study, QR intake in TAA/ QR group corrected the abnormally high liver enzymes compared to TAA group. These results were supported by Hamed *et al* and Afifi *et al*[4,12] Quercetin has been shown to alter key enzymes in proliferation and signal transduction pathways, including those in the MAPK and Akt families.[32]

Histopathological study of TAA/QR group in the present study showed preserved liver architecture with few fibrous bands. This is matching to Hamed *et al* who depicted preservation of nearly normal hepatic architecture.[4]

In the present work, Sirius Red staining revealed decrease in collagen bands and reduced Ishaq score in QR group (score 1) compared to TAA group (score 5). Li *et al*.,[33] demonstrated thinner collagen septa and more preserved liver parenchyma in mice protected by QR than fibrotic animals and lowered mean fibrosis in QR mice group than fibrotic mice group.

The present study examined collagen synthesis by measuring the levels of expression of TGF-β1 immunohistochemistry, TGF-β1 expression levels were markedly lower in quercetin- than in TAA-treated rats after 8 weeks of TAA injection. Ganbold *et al*.,[34] also reported

significant antifibrotic effect of QR on TGFβ-induced hepatic stellate cells.

In summary, the administration of quercetin after the onset of thioacatamide-induced hepatic injury was found to be helpful in reducing the advancement of cirrhotic and hepatic functional impairment. Therefore, the results of this study indicate that QR should be considered a candidate for hepatotoxicity prophylaxis.

Subsequent studies in the same field are recommended, in addition other studies to assess the curative effects of Quercetin on liver cirrhosis and its impact on improving patient's outcomes.

ABBREVIATIONS

ALT: Alanine aminotransferase, **AP**: Alkaline phosphatase, **ECM**: Extracellular matrix, **EIN**: Egyptian Institute of Nutrition, **H&E**: Hematoxylin and Eosin stain, **HSC**: Hepatic stellate cells, **IL-1**: Interleukin 1, **ip**: Intraperitoneal, **IQR**: Inter quartile range, **Kg**: Kilogram, **LD50**: Lethal dose, 50%, **Max**: Maximum, **MFBs**: Myofibroblasts, **mg**: Milligram, **Mic Mag**: Microscopic magnification, **Min**: Mminimum, ***p***: *p value* for comparing between the studied groups, ***p1***: *p value* for comparing between Group I and each other groups, ***p2***: *p value* for comparing between Group II and Group III, ***p3***: *p value* for comparing between Group II and Group IV, ***p4***: *p value* for comparing between Group III and Group IV, **PDGF**: latelet-derived growth factor, **QR**: Quercetin, **SD**: Standard deviation, **TAA**: Thioacetamide, **TGF-β**: Transforming growth factor beta, **TNF-α**: Tumor necrosis factor.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير الوقائى لمادة الكيرسيتين على التليف الكبدى الناتج عن حقن الثيوأسيتاميد لذكور الجرذان البيضاء البالغة

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المقدمة: يعتبر تليف الكبد مشكلة صحية عالمية، تؤثر على كثير من الناس في جميع أنحاء العالم، رغم ذلك لم يتم حتى الآن تطوير نهج علاجي فعال لهذا المرض.

الهدف: تقييم التأثير الوقائي لمادة الكيرسيتين على التليف الناجم عن الثيوأسيتاميد في الفئران.

مواد و طرق البحث: تم تقسيم عدد٤٠ من ذكور الفئران البالغة إلى أربع مجموعات على النحو التالي: المجموعة الأولى (المجموعة الضابطة) ، تضمنت ١٠ من ذكور الفئران والتي تلقت محلول ملحي ١ مجم / كجم عن طريق الحقن البريتوني. وتضمنت المجموعة الثانية ١٠ من ذكور الفئران والتي تلقت كيرسيتين ٥٠ مجم/كجم يوميا عن طريق الفم، والثالثة تلقت ثيوأسيتاميد ٢٠٠مجم/كجم مرتين أسبوعيا بالحقن البريتوني، والرابعة تلقت ثيوأسيتاميد ٢٠٠مجم/كجم مرتين أسبوعيا بالحقن البريتوني وكيرسيتين ٥٠ مجم/كجم يوميا عن طريق الفم. استمرت التجربة لمدة ثمانية أسابيع. تم التضحية بالفئران بعد ٨ أسابيع بعد وزنها ثم تم أخذ عينات الدم للتحليل الكيميائي الحيوي. ثم معالجة عينات الكبد للتحليل النسيجي والمورفومتري.

النتائج: أظهرت النتايج تأثير وقائي للكيرستين علي التليف الكبدي الناتج عن الثيوأسيتاميد، كما يظهر من زيادة وزن الفئران و تحسن نتائج التحليل الكيميائي و تقليل نسبة ترسب الكولاجين في المجموعه الرابعة مقارنة بالمجموعة الثالثة والتي ظهر فيها التليف الكبدي بشكل واضح علي هيئة ترسبات من الكولاجين والتي كونت عقيدات متغيرة الحجم .

الإستنتاج : إستخدام الكيرسيتين له دور وقائي من التليف الكبدي الناجم عن الثيوأسيتاميد. ولذلك يوصى بدراسات مستقبلية في نفس المجال، و كذلك دراسة التأثير العلاجي الكيرسيتين علي التليف الكبدي.