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Biochemical and Molecular Study on the Beneficial Effect of Solubilized Coenzyme Q10 on Thioacetamide-Induced Liver Fibrosis in Adult Male Rats

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Abstract

The present work aimed to evaluate the combined therapeutic effect of both solubilized Coenzyme Q10 and Silymarin on thioacetamide (TAA) induced liver injury. A total of thirty adult male albino rats, weighing 200±10 g were allocated into five groups. Hepatic injury was induced by Thioacetamide (TAA) 20 mg/ kg b wt for 28 days by intraperitoneal injection twice a week. On the other hand, solubilized Coenzyme Q10 (20 mg/kg) & Silymarin (50 mg/kg) were taken orally for 28 days. Upon using the aspartate and platelet ratio index (APRI) & aspartate aminotransferase-alanine aminotransferase ratio (AAR), the TAA group recording the highest values while treatments using Coenzyme Q10 and or Silymarin could decrease theirs values near the control one. However, solubilized CoQ10 could attenuate liver injury induced by TAA via balanced oxidative stress symbols (GSH, GSSG), NO level, and down-regulation gene expression of fibrotic markers TGF-β, collagen-1α, and, TIMP1 as well as enhanced the expression for MMP2 and cytochrome P 450 (CYP 2E1& CYP 3A2). Collectively, solubilized CoQ10 singly or combined with Silymarin showed higher therapeutic impacts more than or equivalents to Silymarin singly treatment for liver fibrosis induced by TAA.

Keywords: Solubilized Coenzyme Q10 -Silymarin - thioacetamide -liver fibrosis and cirrhosis- fibrotic markers- CYP 2E1-CYP 3A2

Introduction

The world prevalent of chronic liver disease, non-alcoholic fatty liver disease (NAFLD) around 25% in the general population and its incidence about 28-86/1000 person years [1, 2]. Worldwide, the liver disease accounts for nearly 2 million deaths each year, 1 million for cirrhosis complications, and 1 million for infectious hepatitis and hepatocellular carcinoma. Cirrhosis is amo-ng the top 20 causes of life-years and years of life suffered due to injury, accounting for 1.6 percent to 2.1 percent of the global burden [3]. Liver cirrhosis is considered a critical stage of chronic liver diseases that leading to liver failure [4]. Liver failure could be induced by exposure to toxic agents, viral infection, or alcohol addiction [5]. Cirrhosis results from the induction of oxidative stress, mitochondrial dysfunction, and depletion of antioxidant status [6]. Cirrhosis is considered to be the advanced stage of fibrosis, hepatic fibrosis can lead to irreversible cirrhosis, and implicates multiple cellular and molecular consequences that eventually result in the accumulation of hepatic collagen and extracellular matrix protein [7], which directly decreasing the hepatic function [8]. The accumulation of extracellular matrix seen in cirrhosis is due to the activation of fibroblasts, which are produced by the activation of hepatic cell precursors, such as hepatic stellate cells [7, 9]. Hepatic disorders can affect the physiological and biochemical functions of the body [10]. Recently, it has been reported that liver fibrosis in human and animal models could be a bidirectional process and can be reversible. So, liver fibrosis therapy management has become more interested and encourages researchers to identify a new safe drug.

Coenzyme Q10 (CoQ10) is an endogenous lipid-soluble benzoquinone that regulates oxidative phosphorylation in mitochondria and subsequent ATP production [11]. CoQ10 is found in the mitochondria, plasma membrane, and functions as an antioxidant either by direct conjugation with reactive oxygen species (ROS) or by regeneration of cellular antioxidants such as tocopherol and ascorbic acid [11, 12]. Tissue or blood levels of CoQ10 represent a pathological marker of the increased oxidative stress in congestive heart failure, coronal artery disease [13, 14], diabetes [15], and liver cirrhosis [16]. In the last 2 decades, significant focus has been paid to the medicinal use of natural ingredients. Plants containing

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advantageous phytochemicals may enhance the requirements of the human body by exerting antioxidative capabilities [17]. Silymarin, an extract from thistle-milk seeds, has been used to treat hepatic disorders for years [18]. It is a polyphenolic flavonoid isolated from milk thistle, which primarily consists of four active compounds. Silibinin represents the most active component in Silymarin (60–70%), which is a flavanone and has several clinical applications as toxic hepatitis treatment, fatty liver, cirrhosis, ischaemic injury, radiation toxicity, and viral hepatitis. It exerts its effect through its anti-oxidation, anti-lipid peroxidation, anti-fibrotic, anti-inflammatory, immunomodulation, and even liver regenerating effects [19].

Thioacetamide (TAA) is the most recommended agent for induction of liver cirrhosis [20] and has been known as a hepatotoxicant via its biotransformation to thioacetamide- sulfoxide (TAAS) by cytochrome P-450 (CYP)-dependent pathway (a CYP2E enzyme), TAAS is subsequently converted to thioacetamide di-sulfoxide, a toxic reactive metabolite [21, 22]. These reactive metabolites covalently bind to liver macromolecules and dramatically increase the production of reactive oxygen species which then induce acute contrilobular liver necrosis [22]. However, the powerful role of mitochondrial antioxidants such as CoQ10 are currently being explored as potential therapeutic agents [23]. Herein, we formulate CoQ10 in high bioavailable nano-emulsions in order to improve its performance and suppose that solubilized CoQ10 through its antioxidant, anti-inflammatory, and apoptotic properties could exert beneficial effects as a hepatoprotective agent in TAA-induced liver damage in rat models. Also, we aimed to evaluate the combined therapeutic effect of both Silymarin and solubilized Coenzyme Q10 on TAA induced liver injury.

Material and methods

Silymarin (82.6%) was donated by Uniphama and supplier by Dongtai Kangnin Vegetable Extraction Co. LTD. TAA and isopropyl myristate (IPM) (HLB = 11.5) were purchased from Sigma-Aldrich, Germany. Coenzyme-Q10 was kindly donated by Mebaco Company, Cairo, Egypt. Tween 80 (HLB = 15) was purchased from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt while, Transcutol® HP (Highly purified diethylene glycol monoethyl ether EP/NF) (HLB = 4) was obtained from Gattefosse Saint-Priest Cedex, France as a gift.

Preparation of solubilized CoQ10 as self-emulsifying nano-emulsion

Solubilized CoQ10 formula was prepared by mixing isopropyl myristate oil (14.3%), tween 80 as surfactant (68.6%), Transcutol as co-surfactant (17.1%) and 30 mg CoQ10 to develop self-nanoemulsifying drug delivery system. This mixture was gently vortexed and placed in the sonicator maintained at 37˚c for 60 min till complete solubilization of drug. This system when dissolved in aqueous medium or in the physiological medium of the body, give solubilized nanoemulsion solution of CoQ10.

Experimental design

The experimental protocol was approved by the Local Animal Care Committee and the experimental procedures were carried out following international guidelines for the care and use of laboratory animals (NODCAR/II/8/2020). Adult male rats, weighing 200±10 g were obtained from the National Organization for Drug Control and Research (NODCAR) Animal House. The animals were kept under normal illumination conditions. They were supplied with standard laboratory chow and water ad libitum and left to acclimatize for 1 week before the experiments. The rats were randomly divided into five groups of six rats each [24]. Group 1: Control animals received vehicle of solubilized CoQ10 (IPM+ Tween 80+Transcutol). Group 2: Thioacetamide (TAA) was intraperitoneally injected TAA, (200mg /kg body weight) dissolved in sterile saline 2 times per week for 4 weeks; [25]. Group 3: received daily 20 mg/kg solubilized CoQ10 [26] orally plus TAA treatment as group two. Group 4: animals received daily 50 mg/kg silymarin [27] by oral administered plus TAA treatment as group two and served as (reference drug). Group 5: received orally solubilized CoQ10 and silymarin (with the previous concentrations) plus TAA treatment. Animals were euthanized on day 29 and blood samples were collected via cardiac puncture. The supernatant serum was separated after blood samples have been left for 30 min to clot, centrifuged at 5000 rpm for 10 min, and stored at −80 ◦C. Platelets count (PLT) was measured by an automated blood cell counter. Subsequently, colorimetric assay kits were used to measure serum aspartate aminotransferase and alanine aminotransferase according to the recommendations of the manufacturer Spectrum Diagnostics; Egypt. Then, the liver was excised, weighed, and subjected for determination of some oxidative stress markers, and several liver genes expression were chosen to be tested.

Indirect serological biomarkers.

The AAR and APRI are two indirect serological biomarkers used for noninvasive diagnosis of hepatic fibrosis. The AAR and APRI were calculated using the following formulas:

AAR = AST (U/L)/ALT (U/L) & APRI = AST (U/L) / ULN *100 / PLT (109/L), where ULN represents the upper limit of normal (= 40U/L) [28].

Oxidative stress markers

Oxidized, reduced glutathione, Sodium nitrite, sodium nitrate and MDA standard (1,1,3,3 tetraethoxypropane; TEP) were obtained from Sigma-Aldrich. The chemicals and reagents used in the current study were of analytical grade.

Determination of tissue MDA (nmol/g tissue) by HPLC:

MDA levels were determined by HPLC according to Karatepe [29]. Supelcosil C18 column (5 µm &250 x 4.6 ID); 82.5:17.5 (v/v) 30 mM monobasic potassium phosphate (pH 3.6), methanol as mobile phase, flow rate (1.2 ml/min) and wavelength 250 nm were applied to Agilent HP 1200 series (USA). MDA standard was prepared by dissolving 25 µl 1,1,3,3 tetraethoxypropane (TEP) in 100 ml of water to give a 1 mM stock solution. Working standard was prepared by hydrolysis of 1 ml TEP stock solution in 50 ml 1% sulfuric acid and incubation for 2h at room temperature.

Determination of tissue GSH (µmol /g tissue) and GSSG (nmol/g tissue) levels by HPLC:

Oxidized and reduced glutathione were detected by HPLC of Agilent HP 1200 series (USA) UV with variable wavelength detector. Bondapak column 30 cm × 3.9 mm C-18 µ was used with flow rate (1ml/min) and wavelength at 190 nm. The used mobile phase contained 0.005 M tetrabutyl ammonium phosphate and 13% methanol; sodium phosphate buffer (0.0025 M; pH 3.5). Samples were determined using oxidized glutathione and reduced reference standard. The results were expressed as µmol/g tissue [30].

Determination of nitric oxide as nitrites and nitrates (mmol/g) by HPLC:

Nitrites and nitrate was estimated by HPLC according to the method of Papadoyannis et al.[31]. A standard mixture of nitrite and nitrate with stock concentration 1mg/ml was used to determine the retention times and separation of the peaks. Nitrite and nitrate concentrations were equal in the mixture solution. Analysis was carried out using Agilent HP 1100 series HPLC apparatus (USA), anion exchange column PRP-X100 Hamilton, 150 x 4.1 mm, 10 µm and a Mixture of 0.1 M NaCl - methanol, at a volume ratio 45:55 as mobile phase. The flow rate of 2 mL/min, wavelength was adjusted to 230 nm.

RNA Extraction and Real-Time PCR (qRT-PCR)

Liver tissues for gene expression were homogenized in chilled and subjected to RNA extraction and purification using Thermo Fisher Scientific Inc. Germany (Gene JET, Kit, #K0732) following the manufacturer's instructions. The yield of total RNA obtained was determined at 260 and 280 nm using Beckman dual spectrophotometer. Gene expression was determined using real-time PCR (Step One, version 2.1, Applied biosystem, Foster City, USA). 1000 ng of the total RNA from each sample was used for cDNA synthesis followed by PCR amplification cycles using SensiFAST™ SYBR® Hi-ROX One-Step Kit, catalog no.PI-50217 V, UK. The thermal cycling profile was 15 minutes at 45ºC for cDNA synthesis followed by 5 minutes at 95ºC for reverse transcriptase inactivation and polymerase activation. PCR amplification 40 cycles were followed which consisted of 15 seconds DNA denaturation at 95ºC, 20 seconds primers annealing at 55 ºC and 30 seconds at 72 ºC for the amplification step. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of the 18s RNA housekeeping gene by the ΔΔCt method. Primer sequences for each gene were given in Table (2).

Statistical analysis

The data obtained from the biochemical analysis of different groups are represented in tables as Mean ± Standard error (X ± SE). The significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) followed by Dunnett t (2-sided) and Duncan. The $P < 0.05$ level of probability was used as the criteria of significance and were carried out using the SPSS-PC computer software package version 21.

Results

Table (1) shows the values of serological biomarkers AAR, and APRI according to different regimes treatments. TAA treated rats had the highest AAR and APRI values of 3.93 and 2.06, respectively at $P<0.05$ compared to control. However, treatment with SQ10, Sily or SQ10+Sily reduced these values significantly compared to the TAA group at $P<0.05$.

Table (1): Serological biomarkers of TAA intoxicated rats with different regimes treatments after four weeks

Parameters	Groups				
	Control	TAA	TAA+ SQ10	TAA+ Sily	TAA+ SQ10+Sily
AAR	1.1±0.02 [a]	3.93±0.07* [b]	1.2±0.01[a]	1.15±0.01[a]	1.19±0.01[a]
ARPI	0.56±0.02[a]	2.06±0.05*[d]	0.68±0.01[b]	0.74±0.02[bc]	0.80±0.03*[c]

Results were expressed as mean ±SE for every 6 rats.

* Significant difference against control at ($P<0.05$) by Dunnett t (2-sided).

Table(2):Primers sequence of all studied genes:

Target gene	Primer sequence: 5`- 3`	Gen Bank accession No.
MMP-2	F: CTATTCTGTCAGCACTTTGG R: CAGACTTTGGTTCTCCAACTT	NM_031054.2
TIMP1	F: ATATTCTGTCTGGATCGGC R: GCTTCGTCACTCCTGTTT	NM_053819.1
Collagen-1α	F: GAACTTGGGGCAAGACAGTCA R: GTCACGTTCAGTTGGTCAA	NM_053304.1
TGFβ	F: TGCGCCTGCAGAGATTCAAG R: AGGTAACGCCAGGAATTGTTGCTA	NM_021578.2
CYP3A2	F:GCTCTTGATGCATGGTTAAAGATTTG R:ATCACAGACCTTGCCAA CTCCTT`	AH005338.2
CYP2E1	F:CTGATTGGCTGCGCACCCTGC R: GAACAGGTCGGCCAAAGTCAC	AF061442.1
GAPDH	F: CCCCTTCATTGACCTCAACTACATGG R: GCCTGCTTCACCACCTTCTTGATGTC	NM_017008.4

Pointed to oxidative stress markers, Table 3 illustrated that TAA caused a significant changes on the oxidative stress markers in all treated groups. TAA caused a significant increase in MDA, NO, and GSSG levels, while it caused a significant decrease in GSH level group compared to control at P<0.05. The other treatments showed significant improvement in MDA, GSSG, GSH levels, and no significant change in NO content when compared to TAA group at P<0.05. Also, it was noticed that there was a significant difference between the values of these markers among the treated groups.

Table (3): Oxidative stress profile of TAA intoxicated rats with different regimes treatments after four weeks.

Parameters	Groups				
	Control	**TAA**	**TAA+ SQ10**	**TAA+ Sily**	**TAA+ SQ10+Sily**
MDA *(nmol/g)*	12.24±0.11a	26.89±0.29*e	14.45±0.19*b	21.27±0.63*d	18.82±0.43*c
GSH *µmol /g*	2.04±0.01d	1.01±0.02*a	2.08±0.03d	1.17±0.02b	1.76±0.08c
GSSG *nmol/g*	0.59±0.01b	1.31±0.04*d	0.35±0.01*a	0.85±0.01*c	0.4±0.02*a
NO *mmol/g*	0.37±0.03a	0.57±0.04*b	0.48±0.01b	0.51±0.04*b	0.49±0.01*b

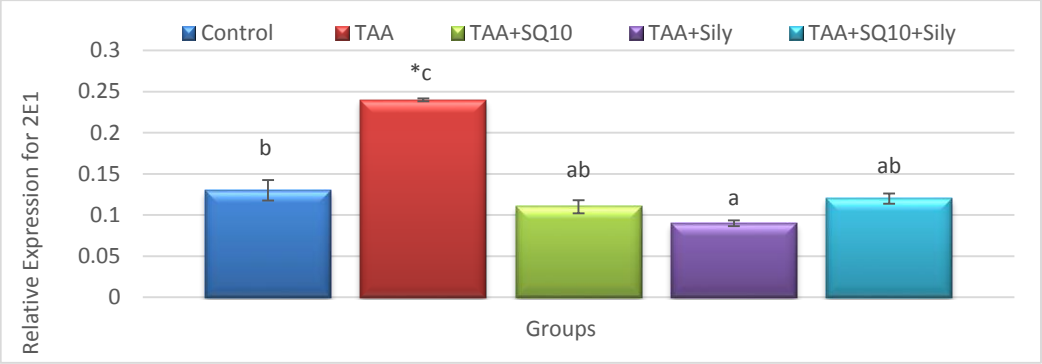
Results were expressed as mean ±SE for every 6 rats.
* Significant difference against control at (P<0.05) by Dunnett t (2-sided).
Groups not sharing a common letter mean significantly different at (P < 0.05) according to Duncan.

As shown in Figure (1), TAA showed significant upregulation in the expression of Cyp 2E1 in rats' livers when compared to the control group. While, the other treatments showed significant down regulating in Cyp 2E1 gene expression compared to TAA group with the priority of (TAA+Sily) treatment than single ones. On the other hand, (TAA+SQ10) and (TAA+SQ10+Sily) treatments equally improved Cyp 3A2 gene comparing to TAA treated group at (P<0.05). Also, we noticed that silymarin treatment showing no significant change towards 3A2 expression as compared to TAA treatment (Figure, 2).

Comparing TAA effect on the fibrotic markers TGF-β, collagen-1α, MMP2, and TIMP1 gene expression in rats' livers (Figures: 3-6), TAA treated animals showed a significant upregulation in TGF-β, collagen-1α, and TIMP1 gene expression associated with a significant down regulation in MMP2 gene expression versus the healthy control group ($p < 0.05$). On the other hand, combined treatments showed a significant down-regulation in TGF-β, collagen-1α and TIMP1 gene expression with the priority of SQ10+Sily treated group when compared to the TAA group as shown in Figures 3, 4&5. Regarding TIMP1 gene expression,

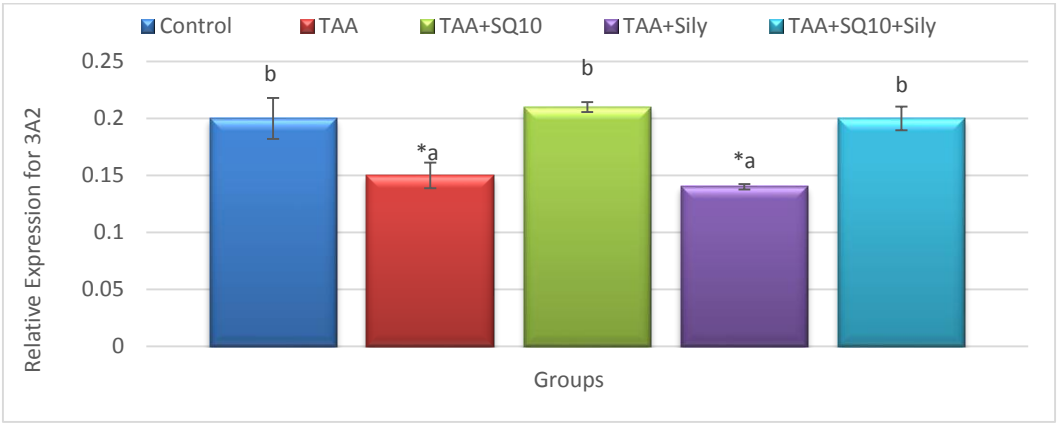
treatment with either SQ10 or Sily showing more down regulation response than SQ10+Sily against TAA group at p＜0.05.

Figure (1): Relative Expression for CYP 2E1 of TAA intoxicated rats with different regimes treatments



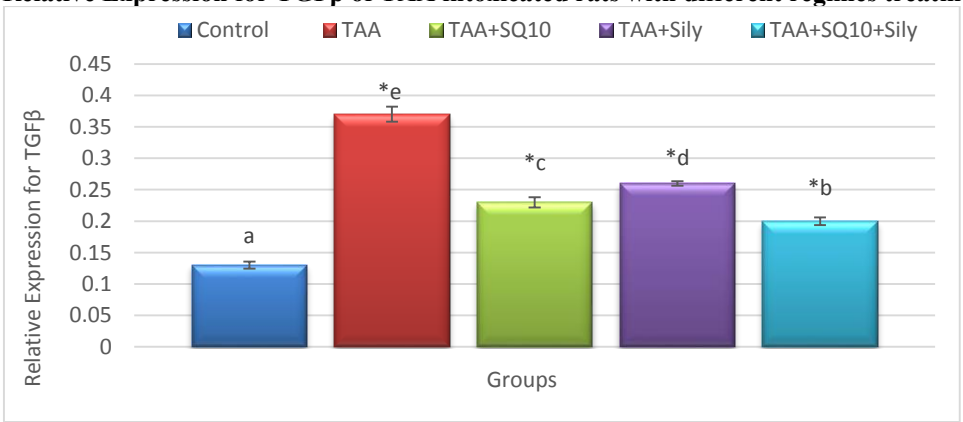
Results were expressed as mean ±SE for every 6 rats.
* Significant difference against control at P<0.05.
Groups not sharing a common letter mean significantly different at P < 0.05

Figure (2): Relative Expression for CYP 3A2 of TAA intoxicated rats with different regimes Treatments



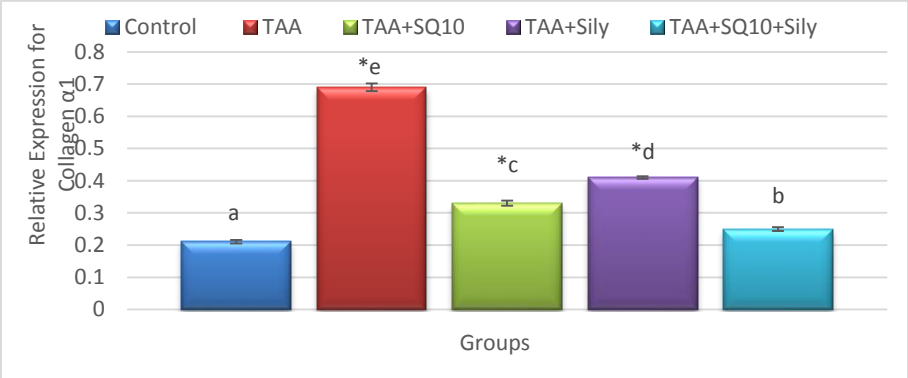
Results were expressed as mean ±SE for every 6 rats.
* Significant difference against control at P<0.05.
Groups not sharing a common letter mean significantly different at P < 0.05.

Figure (3): Relative Expression for TGFβ of TAA intoxicated rats with different regimes treatments



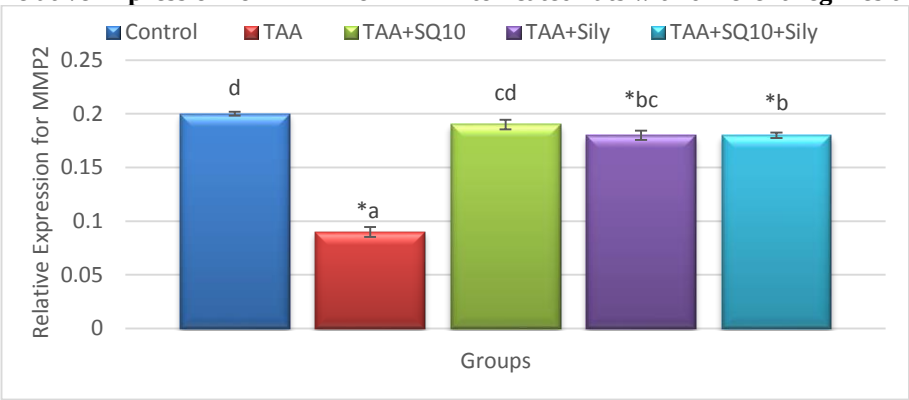
Results were expressed as mean ±SE for every 6 rats.
* Significant difference against control at P<0.05.
Groups not sharing a common letter mean significantly different at P < 0.05.

Figure (4): Relative Expression for Collagen α1 of TAA intoxicated rats with different regimes treatments



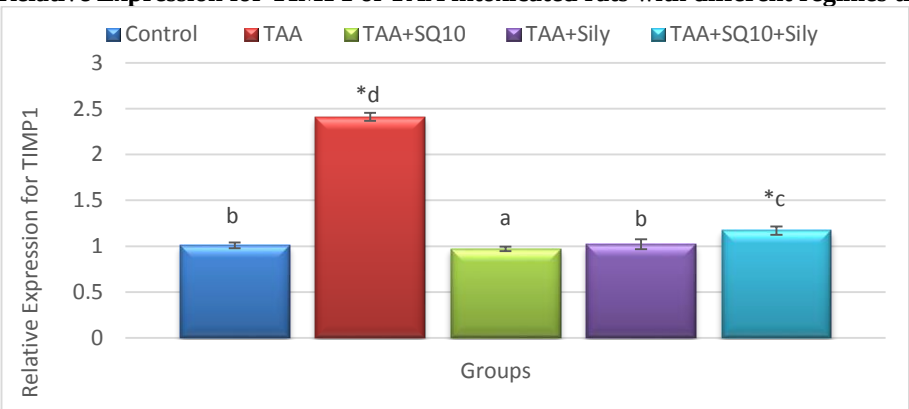
Results were expressed as mean ±SE for every 6 rats.
* Significant difference against control at P<0.05.
Groups not sharing a common letter mean significantly different at P < 0.05.

Figure (5): Relative Expression for MMP2 of TAA intoxicated rats with different regimes treatments



Results were expressed as mean ±SE for every 6 rats.
* Significant difference against control at P<0.05.
Groups not sharing a common letter mean significantly different at P < 0.05.

Figure (6): Relative Expression for TIMP1 of TAA intoxicated rats with different regimes treatments



Results were expressed as mean ±SE for every 6 rats.
* Significant difference against control at P<0.05.
Groups not sharing a common letter mean significantly different at P < 0.05.

Discussion

Liver fibrosis, a precursor of hepatic cirrhosis, has many possible causes of abnormal liver function. One of these are the hepatotoxic molecules which have the ability to react with cell components and induce hepatic disorders that affect the physiological and biochemical functions of the body. In the current work liver injury model was built using a potent hepatotoxic thioacetamide (TAA, 200 mg/kg, b.w. twice a week) for four weeks.

TAA intoxicated rats showed significant alterations in the level of serum biomarker liver enzymes, where a single dose of TAA causes necrosis along with increasing levels of serum transaminases and bilirubin concentrations in rats [22].

The mean AAR and ARPI values had found to increase gradually from control to TAA groups indicating different stages of fibrosis /cirrhosis as well as introducing the evidence of experimental model achievement. Holmberg et al.[32] reporting that the mean APRI values rose significantly with successive fibrosis levels in agreement with Ansar et al.[33]. This finding might be due to a reduction in AST clearance with increasing in its release as liver fibrosis progress [34]. The mitochondrial injury could responsible for the marked release of AST (mitochondria and cytoplasm) to ALT during advanced liver disease as recorded by Okuda et al. [35]. Moreover, the bio-transformation of TAA precedes oxidative damage associated with liver injury. This had been implicated earlier in our study by the presence of a significant elevation in liver MDA, GSSG, NO and GSH depletion in consistently with a previous studies [36, 37]. A study on the role of the antioxidants enzymes in TAA damage has shown that GSH and SOD decreased markedly in TAA group in Wistar rats [38], while the formation of NO increased in the liver disease as the L-arginine-NO pathway is activated [39].

Traditional herbal medicines are normally occurring, plant-sourced substances with next to zero modern handlings that have been utilized to alleviate sickness within conventional healing practices [40].Treatment with coenzyme Q10 or silymarin preserved the hepatocyte integrity. This finding has evidenced by a reduction in plasma activities of ALT and AST and alleviation in TAA-induced oxidative stress in hepatic tissues by CoQ10 that reduces the hepatic oxidative stress and liver function in rats [41, 42]. In our study, solubilized CoQ10 (20 mg/kg) significantly reduced the levels of serum aminotransferases which may be correlated to the antioxidant effect of CoQ10. It also inhibits hydrogen peroxide or Bax-induced destabilization of mitochondria in mammalian cells [43].

Treatment with Silymarin (50mg/kg) significantly decreased serum AST, ALT which may be attributed to the pharmacological properties of Silymarin. These involve regulating cell membrane permeability and integrity, inhibiting leukotriene, scavenging reactive oxygen species [44]. Silymarin decreased leakage of hepatocellular enzymes ALT and AST into the plasma, decreased serum levels of ALP and lessened the development of liver necrosis and fibrosis [39, 45].

Following our result, Silymarin significantly improve the antioxidants disturbance done with TAA, becuase Silymarin can augment the non-enzymatic and enzymatic antioxidant defense systems of cells by preventing lipid peroxidation and replenishing the reduced glutathione levels [39].Furthermore, Silibinin- the active component- exhibits membrane protective properties and can protect blood constituents from oxidative damage [46].

The consequential loss of functional liver mass, sustaining in organ failure, may be physiologically by increased formation of the extracellular matrix. Indeed, profibrogenic cells (e.g., HSCs and myofibroblasts) activated early after the liver injury to produce extracellular matrix components and hyaluronic acid (an indirect marker for collagen formation) [47]. Both hepatic stellate and Kupffer cells are responsible for the propagation of oxidative and inflammatory responses, with subsequent activation of various fibrogenic mediators [48]. Molecular targets include nuclear factor-kappa B (NF-κB), tumor growth factor-beta (TGF-β), as well as several inflammatory mediators [49], had proposed mainly as a part of the oxidative or inflammatory signaling. As a result, the collagen accumulated as a marked liver fibrosis [50] in parallel with the up-regulation of tissue inhibitor of metalloproteinases1 (TIMP-1) and TIMP-2, which serve as markers of HSC activation [51,52].

TAA-challenged mice showed significant up-regulation of TGF-β, a-SMA, collagen-1 genes, and a considerable decrease in MMP-2 gene expression had reported by Algandaby [36] that resembles our results. In the current study, the anti-fibrotic activity of CoQ10 showed significant down-regulation in TGF-β, collagen-1α, and TIMP1 gene expression associated with significant up-regulation in MMP2 gene expression when compared with gene overexpression resulted from TAA induction.

Choi et al. [53] hypothesized that CoQ10 blocks fibrosis by blocking hepatic stellate cell activation, as the α-SMA expression is a marker of activated stellate cells at the earliest stages of hepatic fibrosis. Solubilized CoQ10 administration potently inhibited α-SMA expression in fibrotic livers. Furthermore, NF-E2 related factor-2 (Nrf2) overexpression itself reduced the basal expression of α-SMA and TGFβ1 [53]. Hence, CoQ10-induced Nrf2 activation may suppress TGF-β1expression and stellate cell inactivation. TGF-β1 inhibits antioxidant enzyme expression through Smad3/ATF dependent Nrf2 inactivation [37]. Silymarin administration

significantly declined liver fibrosis induced by TAA – treatment with a significant improvement in hepatic activities.

Compatible with our study Abdel Aal et al. [54] suggested that silymarin has anti-inflammatory effects may be related to its ability to inhibit NFkB, which contributes to the production of pro-inflammatory mediators such as IL-1 and IL -6, TNF- α, and interferon (IFN)-c [55]. Silymarin also inhibits stellate hepatocyte transformation to myofibroblasts. Myofibroblasts are responsible for collagen deposition in CCl4 -induced hepatic fibrosis [56]. In addition, other studies showing a reduced fibrosis in injured liver tissue after silymarin treatment in chronic models of liver injury [57, 58]. Associated with the suggestion exists in the literature for a cytochrome P450-mediated TAA toxicity. TAA induced liver injury after six weeks treatment was affectedly decrease the hepatic protein expressions of CYP1A2, CYP2C6, CYP2E1, and CYP3A2 to 18, 71, 30, and 21% from the control, respectively [59]. Another study conducted with guinea pigs showed increased hepatic CYP2E1 expression in response to chronic alcohol administration [60]. In line with TAA group in our study, overexpression and activity of CYP2E1 appears to play an important role by the degree of steatosis [61].

Silymarin supplementation ameliorate CCl4 [62] and TAA effect significantly by reducing CYP2E1 expression in our results like Abhilash et al. [60].

Collectively, TAA induced toxicity and liver dysfunction in the current work via elevation of serum liver enzymes, altering gene expression of fibrosis, liver cytochrome, and shifting oxidative stress biomarkers. TAA hepatotoxicity could be related to the generation of Reactive Oxygen Species (ROS) [36-38]. Also, it could be to its breakdown to TAA-S-dioxide, which binds to cellular macromolecules causing cell permeability and calcium uptake alteration. This disturbance in calcium stores constraints mitochondrial activity [63]. Many previous studies proved the including of oxidative stress in hepatic tissues following TAA administration [36-38, 64].

The current work mainly sheds light on the role of CoQ10 supplementation and its ability to reduce TAA hepatotoxicity-induced oxidative stress. SQ10 is the most effective treatment superior to the effect of Silymarin singly or combined (Table 3). Previous studies showed CoQ10 to be of potential use as a treatment in diseases in which oxidative stress is a hallmark and reported its beneficial effects in chronic diseases treatment [65-68].CoQ10 is known for a long time as a potent antioxidant, acting as a primary scavenger of free radicals or in the regeneration of tocopherol [66]. Previously published reports studied the antioxidant capability of its pharmaceutical forms using higher doses and confirmed the oxidative stress suppressing effect of Co Q10 [41,42,65].

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