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SYNERGISTIC EFFECT OF NIACIN AND COENZYME Q10 AGAINST CCL4 INDUCED LIVER FIBROSIS IN RAT MODEL

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ABSTRACT

Liver fibrosis is a pathological disease resulting from chronic liver inflammation worldwide. The present study discusses the efficacy of niacin, coenzyme Q10 (CoQ10) and their combination against liver fibrosis induced by carbon tetrachloride (CCl4) in rats. Silymarin as a reference hepatoprotective drug was also evaluated. Liver fibrosis was induced by intraperitoneal administrations of CCl4 (0.5 ml/kg) / (1:9 v/v) olive oil twice a week for six weeks. The evaluation was done through estimation of oxidative stress markers; malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD). Liver function enzymes; serum aspartate and alanine aminotransferases (AST, ALT) and alkaline phosphatase (ALP), inflammatory marker as tumor necrosis factor alpha (TNF-α) and serum total protein content were also estimated. The work was extend to analyze the histopathological picture of liver. After CCl4 injection, severe liver injury was evident, seen as alterations in the hepatic architectures and drastic changes in all biochemical parameters under investigation. Treatment with niacin, CoQ10, their combination and silymarin attenuated the adverse effect of CCl4 by variable degrees. In conclusion, the synergistic action of niacin and coenzyme Q10 showed a good mirror to the improvement occurred of the measured biochemical parameters and the enhancement of liver architectures.

KEYWORDS: toxicity; fibrosis; niacin; coenzyme Q10; oxidative stress.

INTRODUCTION

Liver plays an important role in regulating metabolism, secretion and storage. It has great efficacy towards detoxifying substances and synthesizing useful principles.[1]

Currently, there are million patients with liver fibrosis all over the world, and most of them undergo hepatitis or viral infections. Liver fibrosis is a result of an imbalance between enhanced extracellular matrix protein (ECM) synthesis and diminished breakdown of connective tissue proteins. Hepatic stellate cells (HSCs) are the primary effector cells in liver fibrosis, where cytokine-mediated activation of HSCs into a myofibroblast-like phenotype is a key event during liver fibrogenesis.[2]

Carbon tetrachloride (CCl4) is one of the most toxins used in experimental induction of liver fibrosis to animals.[3] It is a xenobiotic compound that cause oxidative stress and injury hepatic cells.[4] Many studies established the role of CCl4 metabolism in the liver to the highly reactive substance ($Cl3^{-}$), that initiate cell damage through two different mechanisms which are covalent binding to membrane proteins and enhancement of lipid peroxidation process.[5,6]

Niacin, as nicotinamide, is generated from nicotinic acid in the live[7] and it is one of the most effective compounds in medicinal use aiming to its safety.[8,9] Niacin improves endothelial and blood vessels function and reduces inflammation.[10-12] It is also effective in treatment of brain stroke.[12,13]

Coenzyme Q10 (CoQ10) is a lipid-soluble antioxidant compound. It is responsible for the generation of ATP *via* the oxidative phosphorylation by transferring electrons of the respiratory chain, which exists in the mitochondrial membrane of the liver, heart, kidneys and muscles, where they require certain amount of energy for their biological functions.[14,15]

Silymarin (Sily) is an active lipophilic mixture of bioactive compound found in the extract isolated from the seeds and fruits of milk thistle (*Silybum marianum*).[16] Also, Sily has no adverse effect to any organs, acts as a free radical scavenger agent and has an inhibitory effect on multiple cancer cell lines.[16,17]

Therefore, the aim of the present work is to evaluate the combined effect of niacin and CoQ10 in improving the deleterious action of CCl4 that induced liver fibrosis in rats. The evaluation was done through measuring

oxidative stress and inflammatory markers, liver function enzymes, protein content and hepatic architectures profile referring to silymarin as a reference herbal drug.

MATERIALS AND METHODS

All chemicals were of high analytical grade, products of Merck, Germany and Sigma, USA. Silymarin (hepatoprotective reference drug) was provided from SESDCO, Egypt.

Animals and ethics

Male Wistar albino rats (100-120 g) were selected for this study. They were obtained from the Animal House, National Research Centre, Egypt. All animals were housed in standard plastic cages in an environmentally controlled condition with free access of water and diet. They were kept two weeks for acclimatization before starting any experimental procedures. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt to ensure that animals do not suffer at any stage throughout the experiment.

Doses and route of administration

Administration regimen was twice a week for six consecutive weeks. CCl4 (0.5 ml/kg) was suspended in olive oil (1:9 v/v) and injected intraperitoneally.[18] Niacin was orally administrated at a dose of 40 mg/kg b.wt.[19] CoQ10 was administrated orally at a dose of 10 mg/kg b.wt.[20] Silymarin; a reference herbal drug was orally administered at a dose of 100 mg/kg.[21] Normal control group was orally administrated with 0.5 ml normal physiological saline solution.

Experimental groups

36 male Wistar strain albino rats were used in this study. Animals were divided into 6 groups (6 rats each).Group1 served as normal healthy control rats. Group 2 injected with CCl4. Groups 3-5 forced at the same time with CCl4 and niacin, coenzyme Q10, and their combination, respectively. Group 6 forced with CCl4 and silymarin drug.

Sample preparations

Serum sample: Blood collected from each animal by puncture the sublingual vein in clean and dry test tube, left 10 min to clot and centrifuged at 4°C for 10 min. at 3000 rpm. The separated serum was stored at -80°C for further determinations of liver function enzymes and serum protein content. Liver tissue: was homogenized in normal physiological saline solution (0.9% NaCl) (1:9 w/v). The homogenate was centrifuged at 4°C for 5 min. at 3000 rpm. The supernatant was used for further determinations of oxidative stress and inflammatory markers.

Biochemical assays

Oxidative stress marker; malondialdehyde as a product of polyunsaturated fatty acids oxidation was determined by the method of.[22] Glutathione was assayed by the method of[23] Superoxide dismutase activity was estimated by method of.[24]

Liver function enzymes like aspartate and alanine aminotransferases as well as alkaline phosphatase were estimated by the method of,[25,26] respectively.

Inflammatory marker as tumor necrosis factor-α was estimated by ELISA kit (R&D, Minneapolis, MN, USA) as described by the method of.[27]

Total protein was assayed by the method of.[28]

Histopathological study

Liver slices were fixed in 10% paraformaldehyde and embedded in paraffin wax blocks. Sections of 5 µm thick were stained with hematoxylin & eosin (H&E) and Masson's trichrome, then examined under light microscope for determination of pathological changes.[29]

Statistical analysis and calculations

All data were expressed as mean ± SD of six rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program accompanied with least significance difference between groups (LSD) at $p<0.05$.

% change= [(mean of control – mean of treated)/ mean of control] x 100.

% improvement = [(mean of CCl4 group – mean of treated)/ mean of control] x 100.

RESULTS

Regarding hepatic oxidative stress markers, there was a significant increase in MDA level of CCl4 group by 214.77% referring to the control group. Treatment with niacin, CoQ10, their combination and silymarin exhibited significant decrease by 51.98, 45.66, 57.40 and 39.35 %, respectively, as compared with the CCl4 group (Table 1).

In case of GSH level, CCl4 group showed significant decrease by 51.77% relative to the control group. Treatment with niacin, CoQ10, their mixture and silymarin revealed significant increments by 27.82, 39.64, 52.46 and 28.51% respectively, as compared with the CCl4 group (Table 2).

With respect to the SOD activity, a significant decrease amounted to 47.69% was observed in CCl4 group compared to the control group. SOD activity increased significantly by 55.96, 28.38, 72.30 and 24.10% following the treatment with niacin, CoQ10, their combination and silymarin, respectively as compared with CCl4 group (Table 3).

Therefore, the synergistic action of niacin and CoQ10 recorded the most noticeable effect in ameliorating MDA, GSH levels and SOD enzyme activity.

Regarding to the liver function enzymes, AST, ALT and ALP enzyme activities were significantly increase in CCl4 group by 167.60, 151.87 and 68.65%, respectively as compared to the control group. Treatment with niacin, CoQ10, their combination and silymarin significantly decreased AST level by 44.63, 42.82, 54.77, and 44.18%, respectively as compared with the CCl4 group. In addition, ALT was significantly decrease by 29.89, 37.82, 45.93 and 21.02%, respectively. Moreover, ALP was decreased with the same pattern by 17.71, 18.16, 29.60 and 14.74%, respectively as compared with CCl4 group (Tables 4-6). It is important to notice that the combination of niacin and CoQ10 exhibiting the highest improvement value.

Similarly, TNF-α in the CCl4 group was significantly increase by 169.43 % as compared with the normal control group. Treatment with niacin, CoQ10, their combination and silymarin significantly decreased TNF-α level by 33.90, 26.21, 34.75 and 23.92 %, respectively, as compared with the CCl4 group (Table 7).

Remarkably, the combination of niacin and CoQ10 exhibited the most improvement level in this regard. In the meantime, total protein recorded significant increase in CCl4 group by 25.08% comparing with the control rats, while the aforementioned drugs; niacin, CoQ10, their combination and silymarin ameliorated the total protein content by 10.30, 11.38, 12.73 and 11.65%, respectively (Table 8).

Concerning to the liver histopathological pictures, control healthy rats showed normal hepatic cells structure with normal arrangement in thin plats (Fig. 1, a, b). Injured liver with CCl4 showed loss of hepatic architecture, ballooning of hepatocytes, marked degree of hydropic and steatotic changes, massive necrosis and marked degree of fibrous tissue (Fig. 1, c, d). CCl4 group treated with silymarin showed well-arranged nucleated hepatocytes and mild fibrotic tissue (Fig. 1, e, f). Treatment the injured liver with niacin showed well-formed hepatocytes with no fibrotic tissue and minimal fat vacuoles (Fig. 2, a, b). CoQ10 treatment partly preserved the hepatic normal architecture, mild steatosis was recorded without no sign of fibrosis (Fig. 2, c, d). Treatment with CoQ10 and niacin, in a combination, showed normal hepatic architecture. No hydropic, steatosis or fibrotic changes were seen (Fig. 2, e, f).

Table 1: Effect of treatment with niacin and CoQ10 on MDA level in fibrotic rats liver.

Groups	MDA (µmol/mg protein)	% change	% improvement
Control	1.76 ± 0.05^{e}	----	----
CCl_4	5.54 ± 0.42^{a}	214.77	----
CCl_4 treated with Niacin	2.66 ± 0.20^{cd}	51.13	163.63
CCl_4 treated with CoQ10	3.01 ± 0.10^{bc}	71.02	143.75
CCl_4 treated with Niacin and CoQ10	2.36 ± 0.38^{d}	34.09	180.68
CCl_4 treated with Sylimarin	3.36 ± 0.45^{b}	90.90	123.86

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

Table 2: Effect of treatment with niacin and CoQ10 on GSH level in fibrotic rats liver.

Groups	GSH (µg/gm tissue)	% change	% improvement
Control	584.33 ± 17.15^{a}	----	----
CCl_4	281.81 ± 18.27^{e}	-51.77	-----
CCl_4 treated with Niacin	360.22 ± 15.63^{d}	-38.35	13.41
CCl_4 treated with CoQ10	393.05 ± 17.90^{c}	-32.73	19.03
CCl_4 treated with Niacin and CoQ10	429.66 ± 14.5^{b}	-26.46	25.30
CCl_4 treated with Sylimarin	362.17 ± 15.15^{d}	-38.01	13.75

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

Table 3: Effect of treatment with niacin and CoQ10 on SOD enzyme activity in fibrotic rats liver.

Groups	SOD (µmol /mg protein)	% change	% improvement
Control	16.5 ± 0.75[a]	----	----
CCl_4	8.63 ± 0.41[e]	-47.69	-----
CCl_4 treated with Niacin	13.46 ± 0.75c	-18.42	29.27
CCl_4 treated with CoQ10	11.08 ± 0.48[d]	-32.84	14.84
CCl_4 treated with Niacin and CoQ10	14.87 ± 0.39[b]	-9.87	37.81
CCl_4 treated with Sylimarin	10.71 ± 0.75[d]	-35.09	12.60

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

Table 4: Effect of treatment with niacin and CoQ10 on ALT enzyme activity in rats serum injured with CCl4.

Groups	ALT Unit/L	% change	% improvement
Control	66.83 ± 3.32[f]	----	----
CCl_4	168.33 ± 7.63[a]	151.87	-----
CCl_4 treated with Niacin	118.00 ± 2.00[c]	76.56	75.31
CCl_4 treated with CoQ10	104.66 ± 4.50[d]	56.60	95.27
CCl_4 treated with Niacin and CoQ10	91 ± 2.64[e]	36.16	115.71
CCl_4 treated with Sylimarin	132.94 ± 4.15[b]	98.92	52.95

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

Table 5: Effect of treatment with niacin and CoQ10 on AST enzyme activity in rats serum injured with CCl4.

Groups	AST Unit/L	% change	% improvement
Control	71.13 ± 1.85[d]	----	----
CCl_4	190.35 ± 2.62[a]	167.60	----
CCl_4 treated with Niacin	105.39 ± 4.56[b]	48.16	119.44
CCl_4 treated with CoQ10	108.83 ± 7.87[b]	53.00	114.60
CCl_4 treated with Niacin and CoQ10	86.09 ± 3.46[c]	21.03	146.57
CCl_4 treated with Sylimarin	106.24 ± 4.16[b]	49.36	118.24

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

Table 6: Effect of treatment with niacin and CoQ10 on ALP enzyme activity in rats serum injured with CCl4.

Groups	ALP Unit/L	% change	% improvement
Control	137.24 ± 5[d]	----	----
CCl_4	231.46 ± 3.75[a]	68.65	----
CCl_4 treated with Niacin	190.47 ± 5.27[b]	38.78	29.86
CCl_4 treated with CoQ10	189.42 ± 5.16[b]	38.02	30.63
CCl_4 treated with Niacin and CoQ10	162.93 ± 4.16[c]	18.71	49.93
CCl_4 treated with Sylimarin	197.33 ± 3.51[b]	43.78	24.86

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

Table 7: Effect of treatment with niacin and CoQ10 on TNF-α level in fibrotic rats liver.

Groups	TNF-α (pg/mg protein protein)	% change	% improvement
Control	29.48 ± 2.45[d]	----	----
CCl_4	79.43 ± 2.34[a]	169.43	-----
CCl_4 treated with Niacin	52.50 ± 2.5[c]	78.08	91.35
CCl_4 treated with CoQ10	58.61 ± 2.05[b]	98.81	70.62
CCl_4 treated with Niacin and CoQ10	51.83 ± 1.62[c]	75.81	93.62
CCl_4 treated with Silymarin	60.43 ± 0.90[b]	104.98	64.45

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

Table 8: Effect of treatment with niacin and CoQ10 on serum total protein content in rats injured with CCl4.

Groups	Serum protein mg/ml	% change	% improvement
Control	73.75± 2.63[b]	----	----
CCl_4	92.25± 4.57[a]	+25.08	-----
CCl_4 treated with Niacin	81.75± 3.86[b]	+10.85	14.23
CCl_4 treated with CoQ10	82.75±4.96[b]	+12.20	12.88
CCl_4 treated with Niacin and CoQ10	80.50± 3.70[b]	+9.15	15.93
CCl_4 treated with Sylimarin	81.50± 6.75b	+10.50	14.57

- Data are expressed as mean ± SD of six rats in each group.
- Groups having the same letters are non- significantly different, while those having different letters are significantly different at $p < 0.05$.

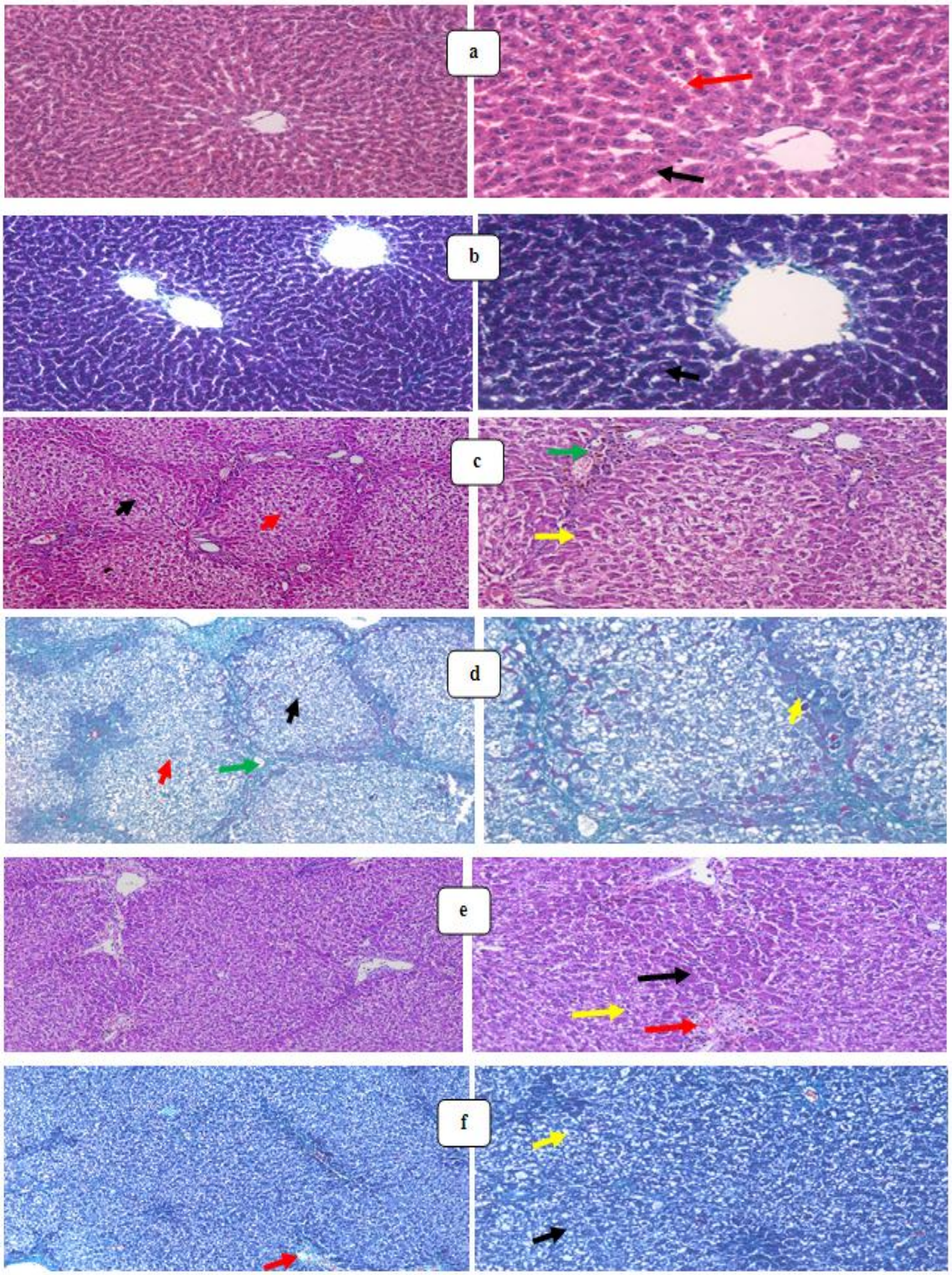

Fig. 1: (a,b) Liver section from control group showed preserved (intact) lobular hepatic architecture with thin plates of normal hepatocytes (black arrow), normal morphological appearance of hepatocytes (hepatic tissue with normal structure and architecture) and mild dilated congested sinusoids (red arrow). (c, d) Liver section of CCl4 group showing stage 4 of fibrosis, loss of hepatic lobular architecture and formation of small (black arrow) and large (red arrow) complete and incomplete hepatic nodules, fibrous tissue (yellow arrow) and portal tract (green arrow). (e, f) Liver section of CCl4 group treated with silymarin showing stage 1 of fibrosis, preserved lobular hepatic architecture with thin plates of normal hepatic cells (black arrow) and mild ballooning of hepatocytes (yellow arrow), portal tract (red arrow). (a, c, e; H&E, x100, x200), (b, d, f; Masson Trichrome,x100, x200).

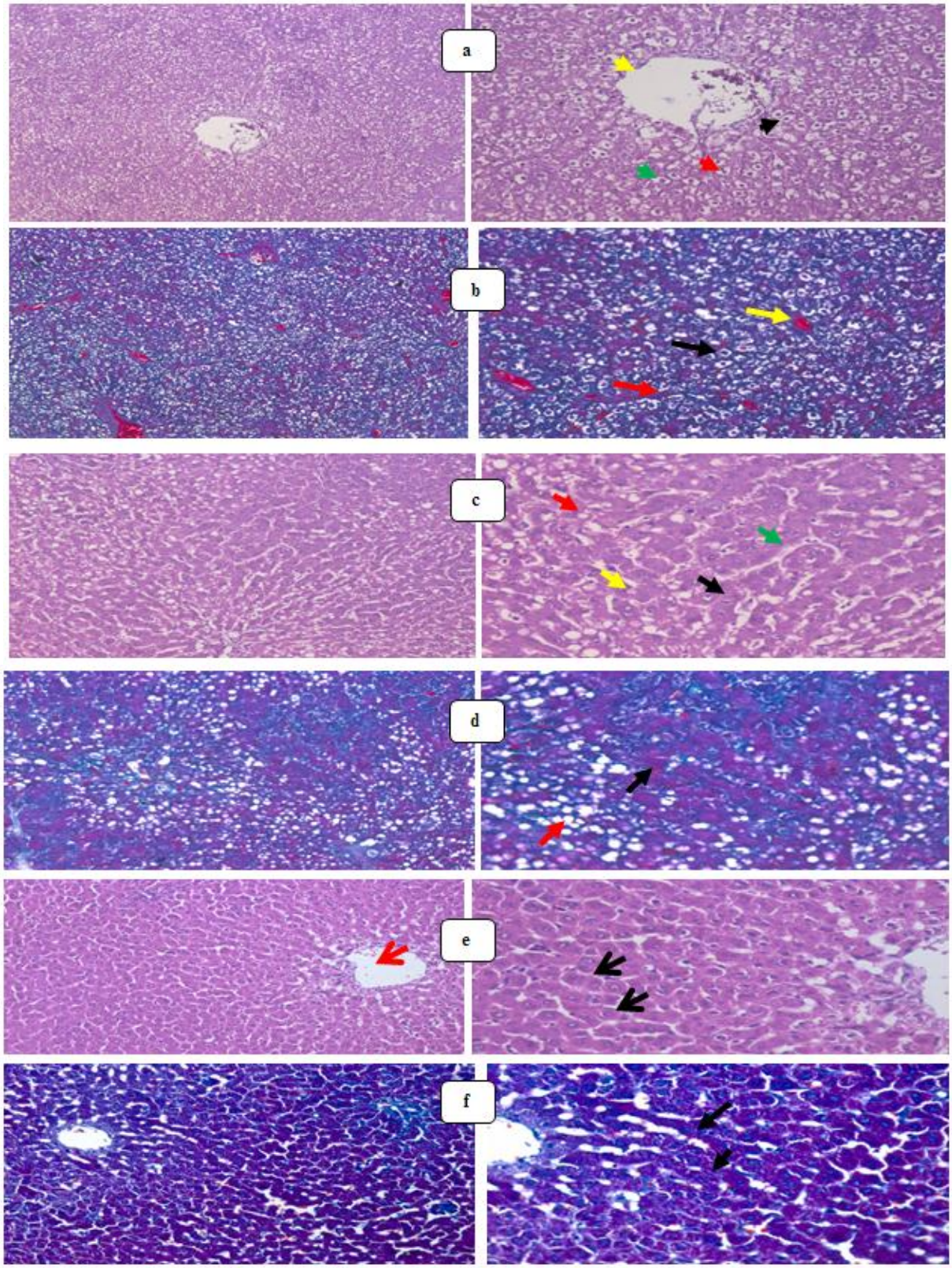

Fig. 2: (a,b) Liver section of CCL4 group treated with niacin showing no sign of fibrosis (stage 0), intact lobular hepatic architecture, ballooning hepatocytes (green arrow), binucleated hepatic cells (black arrow), microsteatotic changes (red arrow) and congestion of the central vein (green arrow). (c,d) Liver section of CCL4 group treated with CoQ10 showing stage zero of fibrosis with preserved lobular hepatic architecture (black arrow), vacuolated hepatocytes (yellow arrow) and steatotic changes (red arrow) with both dilation and congestion of the sinusoids (green arrow). (e, f) Liver section of CCl4 group treated with niacin and CoQ10 showing normal structure of hepatic tissue, the hepatocytes arranged in thin plates (black arrow) with normal central vein without dilation or congestion (red arrow) (a, c, e; H&E,x100, x200), (b, d, f; Masson Trichrome,x100, x200).

DISCUSSION

Chronic liver damage induced by CCl4 in rats produces severe changes in biochemical parameters as well as the histological patterns that resemble human liver cirrhosis. CCl4 alter the lipid peroxidation process and the antioxidant enzyme activities that leads to generation of free radicals.[30] Free radicals bind to DNA, lipids, proteins or carbohydrates, leading cell necrosis and excessive deposition of collagen in liver.[1,31] Evidence suggests that the enzymatic and nonenzymatic systems have been developed by the cell to attenuate the reactive oxygen species (ROS). Therefore, ROS affects the antioxidant defense system, reduces GSH content, decreases SOD enzyme activity and enhances malondialdehyde level.[32] In agreement with these observations, we recorded the same pattern of antioxidant changes in CCl4 treated rats that may be due to the alteration of the antioxidative enzymes.

On the other hand, oxidative stress biomarkers were ameliorated after supplementation with niacin and CoQ10. This may be due to the antioxidant properties of niacin that prevents lipid peroxidation, attenuates the oxidative injury and protects against free radical accumulation.[33-35]

In the present study, the hepatotoxicity of CC14 was confirmed by a significant elevation of AST, ALT and ALP. This may be due to release of these enzymes from the cytoplasm to the blood stream after cellular damage.[36] We observed significant decrease in serum AST, ALT and ALP concentrations in CoQ10 and niacin treated group. CoQ10 may have therapeutic effects against liver damage possibly through its antioxidant, anti-inflammatory and anti-apoptotic actions.[37] In the meantime, niacin restores the altered hepatic enzymes may be due to its antioxidant potential and apoptotic activity together.[38]

Inflammation is associated with hepatic fibrogenesis. CCl4 metabolites interact with hepatocytes and cause parenchymal cells necrosi. It activates inflammatory mediators, including tumor necrosis factor-alpha (TNF-α) (39). TNF-α stimulates the hepatic fibrosis and produces extracellular matrix component (ECM) by activation the hepatic stellate cells (HSC) and fibrogenic tumor gross factor alpha (TGF-β1) from Kupffer cells (40). In agreement with our results, it was observed that supplementation of CoQ10 or niacin reverted these changes.[41] Moreover, it had been postulated that the antioxidant effect of CoQ10 resulted from inhibiting oxidative stress and blocking caspase-3-dependent cell death pathway.[42] Niacin was also found to inhibit TNF-α through down regulating nuclear transcription factor-κB signaling pathway.[43]

The excess deposition of extracellular matrix proteins affect the normal architecture of the liver, alters its normal function and leading to histopathological disturbance.[44] The most remarkable pathological features of CCl4 -induced hepatotoxicity, and in line with our results, are hepatocytes degeneration, fibrosis, steatosis and cellular infiltration.[1] Treatment with silymarin, CoQ10 and niacin recorded variable degree of histological improvement. Silymarin has anti-fibrotic and anti-inflammatory effects, inhibits activation of stellate cells through the over expression of TGF-β1 and mast cells stabilization.[45]

In conclusion, treatment with the combination of niacin and CoQ10 improved the biochemical and the histopathological alterations in fibrotic liver after CCl4 intoxication in rats. This improvement might be due to the synergistic antioxidant and anti-inflammatory effects of both niacin and CoQ10. Eventually, further studies are recommended to support the use of niacin and CoQ10 as a dietary supplement in liver fibrotic conditions.

Conflict of interest

The authors declared no conflict of interest.

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