INTRODUCTION

Liver fibrosis is a reversible wound-healing process that occurs in almost all patients with chronic liver diseases. The sustained liver injuries in chronic liver diseases cause multiple cells and cytokines act in a dynamic and interactive way to comprise the mechanisms of fibrogenesis. Though early stage of fibrosis is usually silent in symptoms; progression to cirrhosis may cause almost all kinds of hepatic complications, including portal hypertension, ascites, hepatic encephalopathy, and impaired metabolic disturbance, therefore, it is largely responsible for mortality of hepatopathy [1,2]. Meanwhile, liver fibrogenesis is orchestrated by a heterogeneous population of profibrogenic myofibroblasts, the majority originating from hepatic stellate cells (HSCs), following a process termed “activation”. Other sources of fibrogenic cells have recently been implicated, including portal fibroblasts and cells derived from the bone marrow [3].

On the other hand, carbon tetrachloride (CCl₄) is a potent hepatotoxin, and a single exposure to it can rapidly lead to severe centrilobular necrosis and steatosis [4]. In general, CCl₄ is metabolized by microsomal mono-oxygenase system (cytochrome P450 2E1) to its active metabolite, this process results in the fragmentation of the lipid peroxide radicals, lipid hydroperoxides and other products, each acting like an...
active oxidizing agent [5]. Furthermore, these processes are immediately followed by the infiltration of inflammatory cells and release of various cytokines and growth factors. Thus, lipid peroxidation caused by free radicals of CCl₄ metabolism plays a vital role on the CCl₄-induced liver injury [6]. In this aspect, the level of tumor necrosis factor (TNF)-α consistently rises during the ongoing CCl₄-induced liver toxicity [7]. It is well-known that TNF-α stimulates the secretion of cytokines such as transforming growth factor (TGF)-β which is also an important activator of HSCs in the course of hepatic fibrogenesis [5].

Vitamin C is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxide damage. It effectively scavenges singlet oxygen, superoxide, hydroxyl, water soluble peroxyl radicals and hypochlorous acid. It is also reported to be an excellent source of electrons and, therefore, can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their activity [8]. Meanwhile, vitamin E, a potent peroxyl radical scavenger, is a chain-breaking antioxidant that prevents the propagation of free radicals in membranes and plasma lipoproteins [9].

L-glycine is a simple, non-essential, and relatively non-toxic amino acid. Current evidences have shown that glycine provides significantly cytoprotective and anti-inflammatory activities. Its beneficial effects do not depend on its metabolism or its influence on cellular energy metabolism, but depend on its special structural characteristics, which glycine shares with other agents such as alanine, taurine, and strychnine (which appears to have similar beneficial effects at high concentrations) [10]. These beneficial effects have been found in a variety of tissues and organs including the liver [11].

Based on the abovementioned facts, the present study aimed to investigate the possible protective effects of both of vitamin C plus E combination and glycine alone on liver fibrosis induced by CCl₄.

**MATERIALS AND METHODS**

**Chemicals**

All chemicals used were of analytical grade purity and needed no further purification. Kits for the determination of serum alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities, as well as kit for determination of serum albumin, were purchased from BioSystem (Barcelona, Spain). CCl₄ was purchased from Aldrich (Milwaukee, WI, USA), nicotinamide adenine dinucleotide phosphate, glycine, glutathione (GSH), p-nitrophenol, p-nitrophenyl-β-D-glucuronide, thiobarbituric acid, hydrogen peroxide (H₂O₂), diethylenetriamine pentaacetic acid, pyrogallol, superoxide dismutase standard and malondialdehyde bis(diethyl acetate) (1,1,3,3-tetraethoxy-propane) were purchased from Sigma (St. Louis, MO, USA). Enzyme-linked immunosorbant assay (ELISA) kits for determination of rat serum TNF-α, TGF-β1 and hyaluronic acid (HA) levels were purchased from Bender Med Systems (Vienna, Austria). Vitamin C and vitamin E were purchased from Pharco (Alexandria, Egypt).

**Animals**

Male Sprague-Dawley, with an average weight of 225-250 g, were purchased from Animal House, Medical Technology Center, Medical Research Institute, Alexandria University. They were maintained in 12 h light/dark cycle at constant temperature of 25°C with free access to standard pellet food and tap-water. They were treated humanely according to the National Guideline for animal care.

**Induction of Liver Fibrosis and Treatment**

60 male albino rats were divided randomly into four groups (n = 15 each): Group I (normal control); Group II CCl₄; Group III (vitamin E+C); and Group IV (glycine). Except for the normal control group, all rats in the other groups received orally 2 ml/kg body weight CCl₄, dissolved in olive oil (1:1, v/v), twice a week; Group III was daily administrated 200 IU/kg body weight vitamin E and 50 mg/kg body weight vitamin C (oral), respectively; rats in group IV were given daily glycine with an oral dose of 0.6 g/kg body weight.

Nine weeks post CCl₄-treatment; rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture, and the separated sera were kept at −80°C till biochemical investigations were carried out.

**Histopathology**

Liver samples were obtained from the same lobe in all animals and fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

**Biomarkers of Liver Functions**

Serum levels of ALT, AST, GGT, and ALP enzymatic activities, as well as serum albumin concentration, were measured using commercially available kits according to the manufacturer’s instructions. Serum activity level of β-glucuronidase (β-Glase) was also measured [12].

**Biomarker of Hepatic Fibrosis**

ELISA kit was used to measure serum HA level according to the manufacturer’s instructions.

**Biomarkers of Oxidative Stress**

The concentration levels of serum malondialdehyde (MDA) [13] and whole blood reduced GSH [14] were measured. Furthermore, erythrocytes levels of glutathione peroxidase (GPx), [15] superoxide dimutase (SOD) [16] and catalase (CAT) activity [17] were determined.
**Hepatic Fibrosis Factors (TGF-β1 and TNF-α)**

Cytokines concentration levels in the serum samples were measured by commercially available ELISA kits according to the manufacturer’s instructions.

**Statistical Analysis**

Statistical analysis was performed with using SPSS software (version 17.0; Chicago, IL, USA). Parametric data were analyzed statistically by one-way analysis of variance followed by post-hoc test when appropriate. Significance level was defined as P < 0.05.

## RESULTS

### Liver Functions

CCl4 significantly increased serum activities of ALT, AST, ALP, GGT, and β-Glase as compared to control (P < 0.05) [Table 1]. Antioxidant vitamins and glycine caused a significant decrease (P < 0.05) in the elevated activity levels of ALT, AST, ALP, and GGT when compared with that of CCl4-treated group.

CCl4 caused a significant reduction in the concentration level of serum albumin as compared to control rats (P < 0.05). Serum albumin concentration level was significantly increased in antioxidant vitamins and glycine pretreated rats (P < 0.05) as compared to CCl4-treated group [Table 1].

### Biomarker of Liver Fibrosis

CCl4 caused a significant increase in serum concentration level of HA (P < 0.05) when compared to control group. This increase was inhibited by the ameliorative effect of antioxidant vitamins (E and C) (P < 0.05) and glycine (P < 0.05) [Table 1].

### Biomarkers of Oxidative Stress

Serum MDA concentration level was increased significantly in CCl4 treated rats (P < 0.05) as compared to control the group. Reduction of serum MDA concentration was observed in antioxidant vitamins and glycine treated groups (P < 0.05) as compared to CCl4 treated group [Table 2].

CCl4 caused a significant depletion of erythrocytes GSH content as compared to control rats (P < 0.05). While GSH content was significantly increased in antioxidant vitamins treated group (P < 0.05) and glycine (P < 0.05) as compared to CCl4-group [Table 2]. Furthermore, CCl4 caused a significant reduction in the enzymatic activities of erythrocytes GPx, CAT, and SOD as compared to the control group (P < 0.05). These enzymatic activity levels were significantly increased in antioxidant vitamins, and glycine treated group (P < 0.05) as compared to CCl4-group [Table 2].

### Hepatic Fibrosis Factors (TGF-β1 and TNF-α)

CCl4 caused a significant increase in serum levels of TNF-α and TGF-β1 (P < 0.05) as compared to control group [Table 2]. This increase was inhibited by the prophylactic effect of antioxidant vitamins and glycine (P < 0.05) [Table 2].

### Histopathological Results

Normal liver architecture has been observed in the control group [Figure 1]. Histopathological changes, associated with CCl4 intoxication for 9 weeks are shown in Figure 2. Histopathological changes in CCl4 + vitamins E and C group are shown in Figure 3. Histopathological changes in CCl4 + glycine group are shown in Figure 4.

### DISCUSSION

CCl4-induced toxic liver injury which is a well-characterized model for hepatic fibrosis has been extensively performed [6]. CCl4 damages hepatocellular membrane via lipid peroxidation and this is followed by the release of inflammatory mediators from the activated inflammatory cells which are thought to potentiate CCl4-induced hepatic injury [18]. AST and ALT, the aminotransferases in liver cells, are cytoplasmic in nature, but upon liver injury these enzymes enter into the circulatory group.

### Table 1: Mean serum activity levels of AST, ALT, ALP, GGT and β-Glase as well as serum concentration levels of albumin and HA in the different study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4+vitamin C and E</th>
<th>CCl4+glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AST (U/l)</strong></td>
<td>53.5±2.1</td>
<td>568±22</td>
<td>146±5</td>
<td>225±12</td>
</tr>
<tr>
<td><strong>ALT (U/l)</strong></td>
<td>37±2</td>
<td>359±17</td>
<td>157±5</td>
<td>198±8</td>
</tr>
<tr>
<td><strong>ALP (U/l)</strong></td>
<td>151±3</td>
<td>561±19</td>
<td>272±12</td>
<td>345±15</td>
</tr>
<tr>
<td><strong>GGT (U/l)</strong></td>
<td>4.2±0.3</td>
<td>13±0.6</td>
<td>8.1±0.5</td>
<td>10±0.5</td>
</tr>
<tr>
<td><strong>β-Glase (U/l)</strong></td>
<td>1.06±0.06</td>
<td>4.62±0.21</td>
<td>2.67±0.13</td>
<td>3.47±0.16</td>
</tr>
<tr>
<td><strong>Albumin (g/l)</strong></td>
<td>35.5±0.88</td>
<td>21.4±0.79</td>
<td>25.5±0.88</td>
<td>25.4±1.1</td>
</tr>
<tr>
<td><strong>HA (ng/ml)</strong></td>
<td>102±3</td>
<td>343±3</td>
<td>189±9</td>
<td>245±4</td>
</tr>
</tbody>
</table>

P<0.05 when compared with “control”, “CCl4” or “CCl4+vitamin C and E groups; n=15 for each group. CCl4: Carbon tetrachloride, AST: Aspartate transaminase, ALT: Alanine transaminase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase, HA: Hyaluronic acid, β-Glase: β-glucuronidase

### Table 2: Mean concentration levels of serum MDA, TNF-α, TGF-β1 and GSH content as well as the mean levels of GPx, CAT and SOD activity in a different study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4+vitamin C and E</th>
<th>CCl4+glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDA (nmol/ml)</strong></td>
<td>0.45±0.02</td>
<td>1.08±0.05</td>
<td>0.56±0.03</td>
<td>0.65±0.02</td>
</tr>
<tr>
<td><strong>GSH (mg%)</strong></td>
<td>34.3±1.2</td>
<td>12.5±0.8</td>
<td>22.0±1.3</td>
<td>24.8±0.59</td>
</tr>
<tr>
<td><strong>GPx (U/g Hb)</strong></td>
<td>9.5±0.5</td>
<td>3.8±0.3</td>
<td>7±0.3</td>
<td>5.5±0.3</td>
</tr>
<tr>
<td><strong>CAT (U/ml)</strong></td>
<td>3049±100</td>
<td>929±60</td>
<td>2584±130</td>
<td>2403±129</td>
</tr>
<tr>
<td><strong>SOD (ng/g Hb)</strong></td>
<td>14.8±0.8</td>
<td>5.1±0.3</td>
<td>11±0.5</td>
<td>9.6±0.7</td>
</tr>
<tr>
<td><strong>TNF-α (pg/ml)</strong></td>
<td>15.2±0.42</td>
<td>26.9±0.7</td>
<td>18.9±0.45</td>
<td>17.5±0.52</td>
</tr>
<tr>
<td><strong>TGFB-β1 (mg/ml)</strong></td>
<td>0.07±0.06</td>
<td>0.52±0.39</td>
<td>3.53±0.27</td>
<td>3.80±0.17</td>
</tr>
</tbody>
</table>

P<0.05 when compared with “control”, “CCl4” or “CCl4+vitamin C and E groups; n=15 for each group. MDA: Malondialdehyde, TNF-α: Tumor necrosis factor-α, TGF-β1: Transforming growth factor-β1, GSH: Glutathione, GPx: Glutathione peroxidase, CAT: Catalase, SOD: Superoxide dismutase, CCl4: Carbon tetrachloride
CCl₄-induced hepatic fibrosis.

For increased lysosomal fragility during the pathogenesis of the rate of release of lipid peroxidation in the hepatic tissue [22]. Thus, the increased lysosomal enzymes into the blood stream due to the enhanced liver lysosomal stability and the subsequent release of serum hydrolases could trigger cell damage and apoptosis, which in part explains, in part, the observed decrease in the level of serum β-Glase from the lysosomes provided evidence for increased lysosomal fragility during the pathogenesis of the CCl₄-induced hepatic fibrosis.

Both plasma albumin and blood clotting factors were mainly synthesized in the liver. When the chronic liver damage led to fibrosis, the albumin contents dropped and prothrombin time prolonged [23]; the results of the present study exhibited that CCl₄-induced chronic liver lesion in rats and there appeared a decrease of plasma albumin level. Therefore, CCl₄ impairs the capacity of the liver to synthesis albumin, so the protein content of serum decreases in such cases [20]. It should be noted that the metabolism of CCl₄ yields metabolites that are hepatotoxic and cause fragmentation of the endoplasmic reticulum and disruption of ribosomes into subunits with subsequent disengagement of the 40S subunit from mRNA [24]. It has been shown that once the protein is oxidized, it becomes highly susceptible to proteolytic degradation [25]. These findings may explain, in part, the observed decrease in the level of serum albumin in CCl₄ treated rats.

Elevated activity levels of serum β-Glase were observed in rats with CCl₄-induced liver cirrhosis [21] and N-nitrosodimethylamine-induced hepatic fibrosis [22]. The increase in circulating acid hydrolases could trigger cell damage and apoptosis, which in turn raises β-Glase level in the serum. However, the increase in serum β-Glase in advanced fibrosis is mostly due to a decrease of liver lysosomal stability and the subsequent release of lysosomal enzymes into the blood stream due to the enhanced lipid peroxidation in the hepatic tissue [22]. Thus, the increased rate of release of β-Glase from the lysosomes provided evidence for increased lysosomal fragility during the pathogenesis of the CCl₄-induced hepatic fibrosis.

Both plasma albumin and blood clotting factors were mainly synthesized in the liver. When the chronic liver damage led to

system due to altered permeability of the membrane. In the agreement with previous studies, the data of the present work confirm the elevation in serum activity levels of AST and ALT in rats treated with CCl₄ and indicate its hepatotoxicity. Moreover, a significant increase in the serum activity levels of ALP and GGT, ectoenzymes of the hepatocyte plasma membrane [19], was observed in CCl₄ treated animals. This elevation could be attributed to CCl₄-mediated acute toxicity increased permeability of the hepatocyte membrane and cellular leakage [20]. Accordingly, it may be speculative, that ALP and GGT could be markers of hepatocyte damage or hepatic dysfunction.

Figure 1: Paraffin section from normal control liver tissue after 9 weeks showing well-arrayed hepatic cords radiating from the centrally dilated central vein (CV) intervening sinusoids (S) are compressed with no congestion; no steatosis and no fibrosis are identified; (a) H and E, ×200, (b) H and E, ×400

Figure 2: (a) Paraffin section from liver tissue after carbon tetrachloride (CCl₄) intoxication for 9 weeks showing severe fibrosis among hepatic parenchyma intersected by thick fibrous septae (F) forming prominent cirrhotic change (F) with intervening regenerative nodules (N) (H and E, ×200). (b) Paraffin section from liver tissue after (CCl₄) intoxication for 9 weeks showing portal tract (PT) with prominent fibrous collagenic stroma (F) and heavy chronic lymphocytic infiltrate (arrow), bile duct proliferation (Bd) is extensive with thick wall congested hepatic vein (*) (H and E, ×400)

Figure 3: (a) Paraffin section from liver tissue after carbon tetrachloride (CCl₄) intoxication for 9 weeks receiving vitamins E and C showing moderate steatohepatitis (arrow) is evident with mild fibro-cellular thin septae (F) and scattered foci of macro and micro vesicular steatosis (V) (H and E, ×400). (b) Paraffin section from liver tissue after (CCl₄) intoxication for 9 weeks receiving vitamins E and C showing residual infiltration of the portal tract (PT) by chronic inflammatory cells (left arrow) with mild lobular hepatic infiltration (right arrow); no steatosis is evident (H and E, ×400)

Figure 4: Paraffin section from liver tissue after carbon tetrachloride intoxication for 9 weeks receiving dietary glycine showing regression of the thick fibrous bands with residual thin interrupted ones (F) among the moderately steatotic hepatic vacuoles (V). Mild steatohepatitis (arrow) is evident (H and E, ×400)
their activation and proliferation during fibrogenesis would have triggered an increased expression of HA along with other connective tissue components. Most of the newly synthesized hyaluronate is excreted into the extracellular space, of which a fraction can leak into the blood stream, and the process will be accelerated during liver necrosis. In addition, more than 90% of the circulating HA is degraded in the hepatic sinusoidal endothelial cells [29]. During fibrosis, the function of the sinusoidal endothelial cells is impaired due to the capillarization of sinusoids and formation of basement membrane in the space of Disse [30]. Thus, an increase in HA expression with its leakage into circulation as well as a decrease in its rate of degradation due to impairment of sinusoidal endothelial cells may explain the observed elevation in serum concentration level of HA.

The principal causes of CCl₄-induced hepatic damage are lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals [31]. A close relationship has been reported between lipid peroxidation and fibrogenesis in rats in which fibrosis was induced by CCl₄ administration [32]. In the agreement with the previous studies, the herein results point out to the development of oxidative stress in rats treated with CCl₄. The developed oxidative stress is manifested by a significant decline in the endogenous antioxidants viz. blood GSH content as well as the activity levels of GPx, CAT, and SOD associated with a significant increase in serum concentration level of MDA. The antioxidant enzymes SOD, CAT, and GPx constitute a mutually supportive team of defense against reactive oxygen species (ROS) [33]. Depletion of GSH results in enhanced lipid peroxidation, which in turn causes increased GSH consumption.

The major mechanism by which CCl₄ induces rat liver fibrosis is that the major toxic metabolite of it, namely the trichloromethyl radical (•CCl₃), results in a series of lipid peroxidation reactions [34]. It has been stated that lipid peroxidation, free radical-mediated process, and certain lipid peroxidation products induce genetic overexpression of fibrogenic cytokines (e.g. TGF-β1 and platelet-derived growth factor) and increase the synthesis of collagen. Free radical and MDA can stimulate the synthesis of collagen and initiate the activation of HSCs [35]. These activated HSCs secrete excess extracellular matrix (ECM) proteins, predominantly collagen Type I, leading to the accumulation of scar matrix, and ultimately hepatic fibrosis [36].

In CCl₄ group, in accordance with the previous report, a significant increase in serum concentration levels of TNF-α and TGF-β1 when compared to that in the control group. TNF-α, pleiotropic pro-inflammatory cytokine produced rapidly by macrophages in response to tissue injury, has been implicated in liver fibrosis [37]. However, the role of TNF-α on HSCs activation and liver fibrosis is complicated and remains controversial. However, it has been reported that TNF-α enhances the expression of tissue inhibitor of matrix metalloproteinase-1 in hepatic HSCs which in turn leads to the inhibition of extracellular matrix degradation by the matrix metalloproteinase family of enzymes [38]. TGF-β1 is the most potent fibrogenic cytokine in the liver; its expression increases during fibrogenesis and it is the dominant stimulus which induces HSCs to increase ECM synthesis [39].

The histopathological examination in the present study highlights and confirms the biochemical results since it is proved that the liver tissue after CCl₄ intoxication for 9 weeks was showing severe histopathological changes that manifested by severe fibrosis among hepatic parenchyma intersected by thick fibrous septae forming prominent cirrhotic changes with intervening regenerative nodules [Figure 2a]. Portal tract (PT) with prominent fibrous collagenic stroma and heavy chronic lymphocytic infiltration, bile duct proliferation is extensive with thick wall congested hepatic vein [Figure 2b].

In Groups III and IV, treatment with antioxidant vitamins E and C together or glycine alone resulted in an improvement in biomarkers of liver function. A significant reduction in the mean serum activity levels of AST, ALT, ALP, GGT, and β-Glase as compared to that in CCl₄-treated group was observed. Furthermore, a significant increase in mean concentration level of serum albumin and a significant decline in the mean concentration level of serum HA were observed as compared to that in CCl₄ treated group. Moreover, treatment with antioxidant vitamins E and C or glycine improved the oxidative status induced by CCl₄. In this context, a decrease in ROS production, reflected by a significant reduction in the mean MDA concentration level, was accompanied by an increase in the antioxidant capacity level as seen by a significant increase in GSH concentration and GPx, CAT, and SOD enzymatic activity levels. Furthermore, the mean serum concentration levels of TNF-α and TGF-β1 were significantly reduced on supplementation with vitamins E and C together or glycine alone.

It has been reported that vitamin C has hepatoprotective property which in turn is linked to its antioxidantive action. Vitamin C was reported to attenuate hepatic damage induced by some chemical agents especially in animals. It was reported that vitamin C normalized levels of ALT, AST, ALP, blood hydroperoxide, and MDA in liver of CCl₄-intoxicated rats [40].

The hydroxyl group of tocopherol reacts with the peroxyl radical to form the corresponding lipid hydroperoxide and the tocopheryl radical (Vit E-O•). The tocopheryl radical reacts with vitamin C (or other hydrogen donors), thereby oxidizing the latter and returning vitamin E to its reduced state [41]. The interaction of vitamins C and E has led to the idea of “vitamin E recycling,” where the antioxidant function of oxidized vitamin E is continuously restored by other antioxidants.

The synergism between vitamin C and other antioxidants has been reported. One of the visible synergies is between vitamin C and vitamin E. All of these studies have pointed out to the ameliorative effect of the co-administration of vitamin C and vitamin E against hepatic intoxication by different agents [42,43]. Moreover, they have pointed out to the normalization of the liver functions biomarkers such as ALT, AST, and some endogenous antioxidants as a result of vitamin C and vitamin E co-administration.
All of the above-mentioned studies are in agreement with results of the present study where co-administration of vitamin C and vitamin E resulted in an improvement in liver functions, reduction in oxidative stress and effective inhibition of TNF-α production from CCl₄-stimulated Kupffer cells. This form of therapy may be attractive because it down-regulates pro-inflammatory cytokine production without total inhibiting all TNF-α bioactivity, some of which may be beneficial to the host.

For Group III, the histopathological examination showed that antioxidants treatment for 9 weeks ameliorated the necrotic and fibrotic changes caused by CCl₄. Histopathological results showed residual infiltration of the PT by chronic inflammatory cells with mild lobular hepatic infiltration. Moderate steatohepatitis is evident with mild fibrocellular thin septae and scattered foci of macro and microvesicular steatosis [Figure 3].

It is most likely that the cytoprotective role of glycine is attributed to its ability to inactivate the Kupffer cells via glycine receptors [44]. The mechanism involved may be related to inhibition of the release of pro-inflammatory cytokines by Kupffer cells induced by glycine. In vivo and in vitro experiments have found that glycine inhibits the secretion of TNF-α and interleukin-6 in Kupffer cells [45]. All of these together may, in part, explain the improved liver functions and decreased oxidative stress. Accordingly, it has been suggested that glycine may be an effective agent which could provide a future strategy for therapeutic intervention in the treatment of liver injuries induced by activated Kupffer cells.

The present histological examination confirmed the biochemical findings which demonstrated dilated PT with moderate lymphocytic infiltration and mild fibrosis separating parenchymal hepatocytes, diffuse macrovesicular steatotic changes with mild to moderate steatohepatitis [Figure 4].

In conclusion, the present study revealed that co-administration of vitamin C and E or glycine improves the liver functions, oxidative status, and fibrinogenesis activity. The mechanism underlying these manifestations may be not only to their antioxidant properties but also through modulating the pro-inflammatory cytokines pathways. Since more effective new therapeutic options are lacking, patients with serious liver diseases should be encouraged to take vitamin E + vitamin C + glycine supplements, which are safe and affordable.

REFERENCES