

## Inflammation and MMPs in Alcohol-induced Liver Diseases and Protective Action of Antioxidants

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The consumption of alcohol causes several liver-associated diseases all over the world. Alcoholic liver diseases (ALD) include hepatic inflammation, fatty liver, hepatitis, liver cirrhosis and fibrosis and finally hepatocellular carcinoma. Although the cellular, metabolic and biochemical mechanisms for these diseases are quite explicable, the roles of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) are still under investigation. The present review describes the roles and regulation of MMPs and TIMPs in different ALDs along with the involvement of other pathways. This review also summarizes the present knowledge on clinical and experimental trials with different antioxidants that help against alcohol associated liver diseases.

**Keywords:** Alcoholic liver diseases, Antioxidants, Matrix metalloproteinase, Tissue inhibitor of MMPs.

### Introduction

Alcoholic liver disease (ALD) includes a spectrum of serious hepatic diseases worldwide<sup>1</sup>. Alcohol consumption is related with continuous progression of inflammatory responses ranging from fatty liver, alcoholic hepatitis (AH) to fibrosis or cirrhosis to hepatic carcinoma<sup>2,3</sup>. The prevalence of ALD is influenced by many factors, including genetic and environmental and associated risk factors, including obesity, iron overload, concomitant infection with viral hepatitis<sup>3</sup>. Although progression of ALD is not at all linearly proportionate with the amount of alcohol ingestion, at a certain threshold of amount, alcohol establishes early manifestation of cirrhosis rather than dose-dependent<sup>4,5</sup>. There is still debate about the safe amount of alcohol consumption, as variability changes with different population worldwide.

The chances of cirrhosis development increases with time; commonly, it develops after more than 10 yrs of alcohol ingestion with a rate of >60-80 g/day for men and >20 g/day for women. Even drinking at this level develops cirrhosis only up to 41% of the population<sup>6,7</sup>. In a population-based cohort study of around 7000 subjects in two northern Italian communities, only 13.5% patients developed ALD, even with very high (>120 g/day) daily alcohol intake rate<sup>6</sup>. In UK, alcohol-related deaths have been doubled in last 20 yrs; death rates in both sexes and all age groups are increasing. It is evident that women are more sensitive to alcoholic doses and durations to develop ALD and alcohol-related deaths are twice as frequent in women as males<sup>8</sup>. Different types of alcohol consumption also have a significant role in development of ALD, as suggested by a survey in Denmark (included more than 30000 persons) which reported that wine ingestion is associated with less liver diseases, rather than beer or spirit consumption<sup>9</sup>.

Matrix metalloproteinases (MMPs), a family of homologous zinc-dependent endopeptidases play a significant role in extracellular matrix (ECM) remodelling in normal physiology and pathologic conditions, including embryonic development, reproduction, inflammation, chronic wounds, arthritis and cancer<sup>10-13</sup>. MMPs are also associated to liver regeneration and liver diseases<sup>14,15</sup>. They

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**Abbreviations:** ALD, alcoholic liver diseases; AP, activator protein; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; HO, heme oxygenase; IL, interleukin; MMP, matrix metalloproteinase; NAC, N-acetyl cysteine; NFκB, nuclear factor kappaB; NOS, nitric oxide synthase; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; TIMP, tissue inhibitors of MMPs.

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are responsible for turnover of matrix proteins like collagen, gelatin, elastin and fibronectin, as well as non-matrix substrates like growth factors, chemokines and adhesion molecules. They are secreted as latent zymogen form and are activated by proteolytic cleavage. The activities and expressions are regulated at different phases like gene transcription, zymogen activation, enzyme secretion and by their endogenous inhibitors, namely tissue inhibitor of metalloproteases (TIMPs)<sup>16</sup>.

Additionally, MMP activities are fine-tuned by regulation of mRNA stability, translational efficiency, enzyme compartmentalization, cell surface recruitment, substrate targeting, cellular uptake and autolysis. Each TIMP preferentially targets different MMPs, although each TIMP also has a lower inhibitory effect on other MMPs that regulate cellular migration, proliferation, and invasion<sup>17,18</sup>.

This review aims to understand the mechanisms for development of different ALD and their relationships with MMPs. The review discusses about the involvement of MMPs and relevant pathways in different ALDs (Fig. 1) and summarizes the different clinical and experimental antioxidant therapeutics or preventive approaches against ALD.

## Types of liver diseases

### Fatty liver disease

Fatty liver disease, also known as hepatic steatosis, characterizes a type of liver disease, where vacuoles of triglycerides accumulate in liver cells. It develops from various alcoholic and non-alcoholic reasons<sup>19</sup>. Fatty liver is very common (~90%) in people who drink more than 60 g/day alcohol; it is largely symptom-free and occurs within few months of

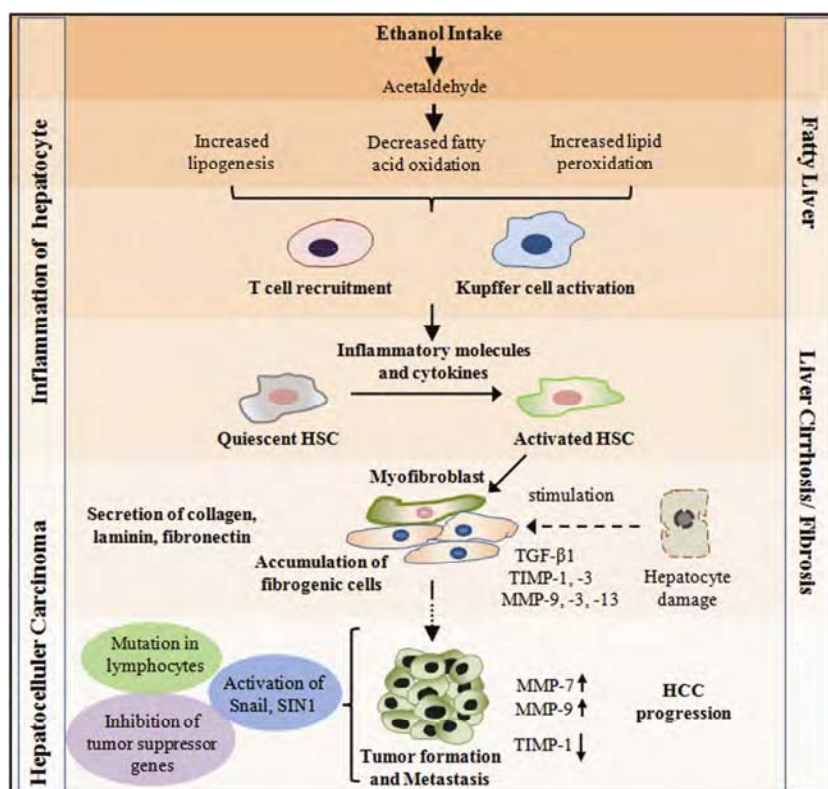


Fig. 1—Cellular mechanism of alcoholic liver diseases [During alcohol ingestion ethanol and its derivative acetaldehyde initiate enhanced lipogenesis and impair beta oxidation that promotes inflammatory responses in liver cells<sup>22,23</sup>. Progressive hepatocyte damage and cytokines activate Kupffer cells and stimulates T-cell recruitment. Elevated inflammatory milieu stimulates residential hepatic stellate cells to trans-differentiate into fibrogenic myofibroblast-like cells. Myofibroblasts migrate to damaged sites and secrete excessive amount of extracellular components that generate hepatic scars and subsequently fibrosis<sup>40</sup>. Prolonged period of alcoholism suppresses tumor suppressor genes and activates mutations in lymphocytes that facilitate the development of hepatocellular carcinoma and further metastasis<sup>58</sup>. MMP activity increases with alcohol-mediated hepatocyte damage and upregulate further with inflammation progression and liver diseases. TIMP regulates MMPs activities; although during severe hepatic damage it fails to control normal MMP/TIMP balance<sup>15</sup>. ECM, extracellular matrix; HSC, hepatic stellate cells; TGF, transforming growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of MMPs; SIN1, smad interacting protein 1, HCC, hepatocellular carcinoma]

regular alcohol intake<sup>20</sup>. Alcoholic fatty liver disease is often the first step in developing or is associated with more serious diseases, such as alcoholic cirrhosis and hepatitis<sup>3</sup>. The pathological signaling starts from oxidative stress, metabolism-related defects and inflammatory cascades to apoptotic responses (Fig. 1). Moreover, ethanol being a source of energy (29.7 kJ/g), displaces normal nutritional pathway, leading to malnutrition.

Alcohol dehydrogenase oxidizes ethanol to acetaldehyde, which further converts to acetate by acetaldehyde dehydrogenase, producing NADPH. Excess NADPH causes a number of metabolic disorders, including increased fatty acid synthesis by activation of sterol regulatory element-binding protein and inhibition of the Krebs cycle and fatty acid oxidation<sup>19,21-23</sup>. The inhibition of fatty acid oxidation favours steatosis and hyperlipidemia. In addition, the microsomal electron transport system also oxidizes ethanol by cytochrome P450 enzymes. The 2E1 isoform of cytochrome P450 (CYP2E1) is induced during chronic alcohol consumption and results in formation of reactive oxygen species (ROS) and increased generation of hydroxyl radicals<sup>24</sup>.

Acetaldehyde-mediated mitochondrial damage aggravates oxidative stress by binding with reduced glutathione and promotes mitochondrial leakage. Also, ethanol uptake significantly increases lipid peroxidation and protein oxidation. Oxidative stress-mediated inflammation causes production of pro-inflammatory cytokines e.g. tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) from Kupffer cells. Destruction of Kupffer cells by gadolinium chloride significantly reduces the increased levels of AST (aspartate aminotransferase), inflammatory markers, fat accumulation and hepatic necrosis during alcohol injury<sup>25</sup>.

Ethanol shows decreased peroxisome proliferator-activated receptor (PPAR)- $\alpha$  activation in the liver, which fails to induce fatty acid  $\beta$ -oxidation, leading to fatty liver<sup>26</sup>. Another study using PPAR- $\alpha$  null mouse has shown similar protective role of PPAR- $\alpha$  against alcohol-induced liver toxicity<sup>27</sup>. Moreover, excessive PPAR- $\gamma$  activity in mouse liver can lead to the development of novel type hepatic steatosis with adipose tissue-specific gene expression and lipid accumulation<sup>28</sup>. Ethanol-induced liver damage causes increased oxidative stress and inflammation, leading to the increased MMP-9 activity. In addition, ethanol enhances nuclear translocation of nuclear factor kappa-B (NF $\kappa$ B) and increases degradation of

inhibitor of NF $\kappa$ B (I $\kappa$ B $\alpha$ ) in hepatocytes<sup>29</sup>. Chronic alcohol enhances the AP-1 mediated signalling pathways in experimental rat liver<sup>30</sup>, which transcriptionally regulates MMPs.

#### **Alcoholic hepatitis**

Alcoholic hepatitis (AH) develops in a subset of ALD patients, ranging from mild to chronic liver injuries, which arise with consumption of large quantity of alcohol over a prolonged period of time<sup>31</sup>. AH develops up to 10-35% of heavy drinkers and about 50% of AH patient leads to hepatic cirrhosis<sup>32,33</sup>. The risk of permanent damage from AH develops among those who continue to abuse alcohol and causes deregulated metabolic symptoms to multi-organ failure and death<sup>34</sup>. Moreover, alcohol and hepatitis C have shown synergistic effects on liver injury, as alcohol may impair immune responses against virus. In a large cohort study, it has been observed that in heavy alcoholic patients with post-transfusion hepatitis C, the risk of cirrhosis development increases about 30-fold<sup>35</sup>.

AH is characterized by increased hepatic inflammation and necrotic responses and Toll-like receptor (TLR)-4 and in association with NADPH oxidase isozyme 4 is involved in ROS generation in neutrophils. Moreover, excessive alcohol consumption changes gut microbial environment, producing more lipopolysaccharide (LPS) that activates Kupffer cells through the CD14/TLR-4 complex to produce ROS via NADPH oxidase<sup>36</sup>. TNF- $\alpha$  induces neutrophil infiltration and stimulates mitochondrial oxidant production in hepatocytes, which are sensitized to undergo apoptosis.

Inhibition of TNF- $\alpha$  activity by administering anti-TNF- $\alpha$  antibody attenuates hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat<sup>37</sup>. Mice deficient in MMP-2, MMP-3 and MMP-9 have shown protection against TNF- $\alpha$ -induced hepatitis and less apoptotic responses<sup>38</sup>. MMP-8 deficient mouse have also shown protection against TNF- $\alpha$  induced hepatitis by reducing hepatocyte death<sup>39</sup>.

#### **Hepatic cirrhosis/Fibrosis**

Hepatic cirrhosis represents development of regenerative hepatic nodules with fibrous scars in response to hepatic damage. It causes hepatocellular dysfunction and increased inter-hepatic resistance to blood flow, which results in hepatic insufficiency and portal hypertension respectively<sup>40</sup>. Histologically, hepatic fibrosis is characterized by vasculated fibrotic

septa that develop hepatocyte islands, without any central vein. The space of Disse is filled with connective matrix and normal endothelial fenestrations of hepatocytes are perturbed. Continuous alcohol intake (>40 g/day) increases the risk to develop cirrhosis by 30% and fibrosis or cirrhosis to 37%<sup>41</sup>. Several studies have also suggested that despite quitting alcohol the chances for develop cirrhosis and fibrosis for alcoholic patients are about 5-15%<sup>42,43</sup>. Alcohol has a threshold effect rather than a dose response effect on mortality due to alcoholic cirrhosis<sup>4</sup>. Peri-venular sclerosis has been identified as a significant and independent risk factor for the progression of alcoholic liver injury to fibrosis or cirrhosis<sup>2</sup>.

Alcohol-induced oxidative stress and damage in hepatocytes influence release of inflammatory molecules and cytokines that activates Kupffer cells and activates T cell recruitment. These inflammatory milieu stimulate resident stellate cells in liver. In chronic injuries, stellate cells differentiate into myofibroblast-like cells and migrate to the injured area. These activated stellate cells act as pro-inflammatory and fibrogenic cells and secrete large amount of ECM proteins to the injured area (Fig. 1). Moreover, TGF- $\beta$  favors the transition of stellate cells and induces the production of ECM protein components. Strategies that inhibit or block TGF- $\beta$  show significant recovery from fibrosis in different experimental models<sup>44</sup>.

As collagenolytic activities regulate the major mechanism of fibrosis resolution, increased interstitial MMPs, including MMP-1, -8 and -13 are responsible for fibrotic development<sup>45</sup>. Moreover, decreased levels of endogenous inhibitors of MMPs, especially TIMP-1 aggravate the fibrosis<sup>46</sup>. Partial degradation of fibrillar collagen leads to altered interaction between activated HSCs and ECM that favours apoptosis. Liver cirrhosis is associated with an early up-regulation of MMP-13 activity which declines in later stages of the disease<sup>47</sup>. In addition, increased gelatinolytic activities (MMP-2, -9) have been observed in liver cirrhosis during experimental bile duct ligation<sup>48</sup>.

Additionally, increased expressions of MMP-2 and -7 mRNA are observed in cirrhosis, compared with healthy controls. Moreover, increased active MMP-2 levels have been observed with an associated upregulation of MT1MMP expression in fibrotic human livers<sup>49</sup>. Advanced stages of hepatic fibrosis are associated with excessive quantitative changes in

ECM components<sup>50</sup>. These stages have more than 6-fold increased ECM components than control persons mainly due to increased synthesis and less degradation of ECM components by upregulating TIMPs<sup>51</sup>. Increased TIMP-1 significantly attenuates spontaneous resolution of liver fibrosis by reducing MMP activities and apoptosis in HSC<sup>52</sup>.

#### Hepatic cancer

Hepatocellular carcinoma (HCC) is one of the most frequent primary tumors worldwide and mainly develops due to very long-term alcohol consumption. In North America and European nations, HCC develops from more than 80% cases of cirrhotic liver, whereas about 50% in Asian countries<sup>53</sup>. Alcoholic patients with history of cirrhotic hepatitis C virus (HCV), rather show increased chances to develop HCC (13.3%, 41.3% and 80.7%, respectively) with time (3, 5 and 10 yrs, respectively) when they continue alcohol consumption (120 g/day)<sup>54</sup>. The relative risk (RR) for development of HCC is 7.3, when the rate of alcohol consumption is more than 80 g/day<sup>55</sup>.

The mechanism of ethanol-induced carcinogenesis is still under investigation. Although, ethanol is still not considered as carcinogen, its first metabolite, acetaldehyde is a potent carcinogen. In experimental models, inhalation of acetaldehyde has shown development of nasal adenocarcinoma and squamous cell carcinoma<sup>56,57</sup>. It interferes with DNA synthesis and causes point mutations in hypoxanthine phosphoribosyltransferase 1 (HPRT1) locus of human lymphocytes and induces gross chromosomal aberrations. Moreover, ethanol and acetaldehyde inhibit adenosyl-L-methionine synthesis that helps cancer progression by decreasing methyl transfer to tumor suppressive genes and their subsequent transcription<sup>58</sup>. It is evident that increased MMP activities are involved with cancer progression<sup>11</sup>.

In HCC, upregulation of mesenchymal transcription factors, snail and smad interacting protein 1 (SIN1) are involved with increased MMP activities, increasing cancer invasion<sup>59</sup>. Increased MMP-7 activities are found to have significant correlation with hepatic cancer, as active MMP-7 is involved in metastasis<sup>60</sup>. Moreover, MMP-2 and -9 expressions in bile can be used as markers for liver metastasis in colorectal cancer<sup>61</sup>. Inhibition of MMPs by TIMPs, more specifically TIMP-1 has regulation against metastasis, as spontaneous and experimental

metastasis in liver is reduced during *Timp-1* overexpression and is increased in mice that exposed to antisense *Timp-1*<sup>62</sup>.

### Antioxidant treatments

Alcohol causes increased oxidative damage and inflammatory responses in hepatic tissues, both in acute and chronic diseases. Attenuation of oxidative stress using different antioxidant treatments has been proved to be effective in reducing alcoholic damage<sup>63</sup>. Moreover, antioxidant enzymes have been shown to protect against alcoholic damage and induction of SOD (both CuZn and Mn) has protective effect on alcoholic injuries<sup>64</sup>. Furthermore, CuZnSOD deficient mice have shown extreme sensitivity to moderate ethanol consumption, increasing oxidative stress through formation of peroxynitrite, protein carbonyls, lipid peroxidation and decreased mitochondrial GSH<sup>65</sup>.

Potent antioxidant N-acetyl cysteine (NAC) attenuates oxidative stress in rats. NAC treatment (1 g/kg b.w.) diminishes oxidative stress by increasing antioxidant enzymes and restoration of oxidant/antioxidant balance (reflected by lower levels of transaminases, ALP and GGT)<sup>66</sup>. Moreover, another study has also reported that antioxidants like NAC and dimethyl sulfoxide (DMSO) (although DMSO is toxic and cannot be used as therapeutics) have significant potential action against oxidative stress and proMMP-9 expression. NAC (100 mg/kg b.w.) and DMSO (3 mg/kg b.w.) treatments decrease proMMP-9 expression by ~88% and ~75%,

respectively during alcoholic liver damage in mice<sup>29</sup>. Our group has also observed similar results of decreased cell viability and increased nuclear condensation, when HepG2 cells are treated with ethanol (Fig. 2). Pretreatment with different antioxidants e.g. catechin, quercetin, curcumin and melatonin have shown protection against alcoholic damage that rescued cell viability (Fig. 2).

### Melatonin

Being a physiological regulator and antioxidant, melatonin has also shown protective effect against ALD. Melatonin protects against alcoholic liver injury by attenuating oxidative stress, inflammatory and apoptotic responses. It significantly attenuates the increased level of serum aminotransferase, reduces the severe extent of hepatic cell damage, steatosis and the immigration of inflammatory cells, but has no effects on hepatic expression of lipogenic gene<sup>67</sup>. Also, melatonin decreases serum and tissue TNF- $\alpha$  levels, tissue lipid peroxidation, neutrophil infiltration and apoptosis of hepatocytes<sup>29</sup>.

In addition, melatonin also dose-dependently arrests proMMP-9 upregulation both at the level of secretion and synthesis. The inhibition of proMMP-9 involves mechanism via decreased pro-inflammatory cytokine (e.g. TNF $\alpha$ , IL-1 $\beta$ ) levels<sup>29</sup>. In addition, melatonin reduces MMP-9 activity by upregulation of TIMP-1 expression, while TIMP-2 expression remains unaltered<sup>29</sup>. The upregulation of TIMP-1 at protein level, as well as mRNA level indicates

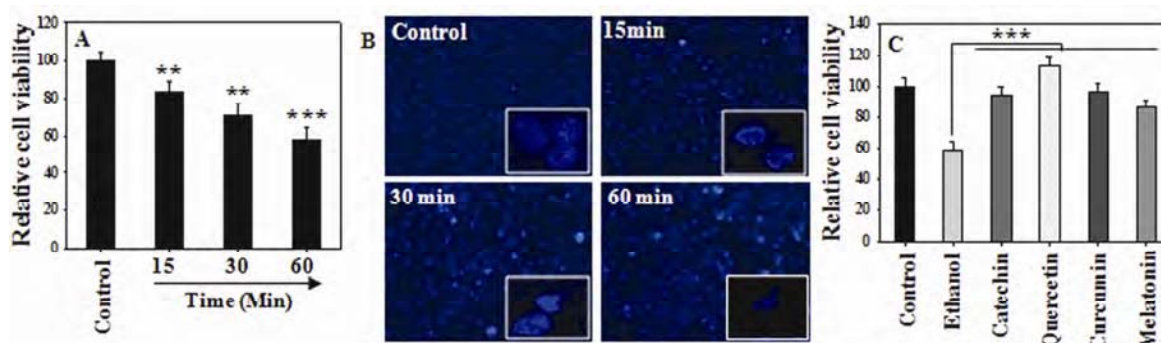


Fig. 2—Effect of ethanol and different antioxidant treatments on HepG2 cells [(A) Cells were treated with 500 mM of ethanol for 15, 30 and 60 min. Cell viability was checked by MTT assay. Briefly, equal numbers of cells ( $2 \times 10^4$ ) were incubated with MTT for 3.5 h and then dissolved in DMSO. Reading was taken at 550 nm using ELISA reader; (B) Nuclear condensation for ethanol treatment was observed at given time points using DAPI staining. Inset higher magnification of nucleus; and (C) For antioxidant treatment, cells were pretreated for 30 min with 50  $\mu$ M of different antioxidants (catechin, quercetin, curcumin, melatonin) prior to the 1 h ethanol treatment. Cell viability was measured by MTT assay. Values expressed as mean  $\pm$  SEM.  $p < 0.05$  was accepted as level of significance; \*\*\*highly significant  $p < 0.001$ ; \*\*significant  $p < 0.01$ ; \*less significant  $p < 0.05$ ]

another beneficial role of melatonin in balancing MMP-9/TIMP-1 ratio, while protecting ALD<sup>29</sup>.

#### Vitamin E

Almost all types of vitamins are found insufficient in alcohol abusers, thus restitution of vitamins is essential for patients. The Institute of Medicine of the US National Academy of Sciences considers only vitamins E and C to be dietary antioxidants, defined as "a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species on normal physiological function in humans". Vitamin deficiency causes from less intake, absorption of nutrients and interference of alcohol in conversion into metabolically active forms.

Vitamin E is reported to have antioxidant activity and prevents the lipids from oxidation<sup>68</sup>. It (100 mg/kg b.w.) decreases oxidative stress by attenuating lipid peroxidation and protein oxidation. It also decreases TNF- $\alpha$  expression, along with proMMP-9 expression in liver tissue of rodent<sup>69</sup>. A randomized clinical study has shown that vitamin E can improve obesity-related liver dysfunction for children, who are unable to adhere to low-calorie diets<sup>70</sup>. Another randomized clinical study has revealed that it improves serum hyaluronic acid, although no beneficial effects is observed on liver function tests in patients with mild to moderate alcoholic hepatitis<sup>71</sup>.

#### Vitamin C

Liver diseases are highly related with reduced ascorbic acid levels in leukocytes. Significant reduced levels of vitamin C have been observed in ALD patients, especially in primary biliary cirrhosis patients<sup>72</sup>. Moreover, vitamin C at a dose of 50-100 mg/kg b.w. ameliorates increased of serum ALT level and hepatic iron overload. Pretreatment of ascorbic acid increases the expression of hepcidin and decreases transferrin receptor 1 and ferroportin 1 expression that protects against alcoholic liver injuries<sup>73</sup>. Ascorbic acid supplementation causes faster restoration of reduced glutathione content in the regression of alcohol-induced hepatotoxicity in male guinea pigs<sup>74</sup>.

#### Curcumin

Curcumin, a polyphenol compound, isolated from turmeric has been used as a hepatic protector in Indian civilization from centuries. Being an antioxidant, it

can stimulate nuclear factor erythroid-2-related factor 2 (nrf2), a transcription factor that regulates different oxidative stress-mediated pathways. Curcumin at the dose of 400-1200 mg/kg b.w. in rats shows significant decrease in oxidative stress and inhibits apoptotic responses in alcoholic liver through NF $\kappa$ B-mediated pathway<sup>75</sup>.

Curcumin is also reported to prevent hepatic cirrhosis development by anti-inflammatory pathways; however, it has not shown any significant improvement when treated on pre-existing cirrhosis<sup>76</sup>. Also, it has no significant effect on PDGF-induced MMP-2 activity and TIMP-1 and -2 expressions on HSC-T6 cell line. However, it shows reduced activated stellate cells and enhances expression of PPAR- $\gamma$  that disrupts TGF- $\beta$  signaling and collagen accumulation<sup>77</sup>.

#### Catechin

A flavonoid from tea and cocoa, catechin has also been reported to have antioxidant property that protects against ALD. Catechin pretreatment at a dose of 40 mg/kg b.w. for 6 weeks ameliorates the alcohol-induced liver injury by inhibiting NF $\kappa$ B activation. It also attenuates inflammatory pathways (including TNF- $\alpha$ , NO) and activates antioxidant enzymes<sup>78</sup>. Moreover, inflammatory micronucleated cell count is remarkably decreased with catechin supplementation that restores protection against alcohol-induced toxicity. However, a randomized double-blind clinical trial has demonstrated that 3-palmitoyl-(+)-catechin at a dose of 1500 mg daily for 3 months fails to produce statistically significant clinical, biochemical or histological benefit in patients with biopsy-proven alcoholic liver disease<sup>79</sup>. Another randomized double-blind clinical trial has shown similar results, with no significant benefit; although both reports have documented significant reduction in alcohol consumption during therapeutic studies<sup>79,80</sup>.

Green tea extract and cocoa, the important sources of antioxidant polyphenols have also been reported to block ethanol-induced lipid peroxidation and TNF- $\alpha$  production, demonstrating that simple dietary antioxidants are able to prevent early alcohol-induced liver injury, most likely by preventing oxidative stress<sup>81</sup>. Moreover, green tea flavanol epigallocatechin-3-gallate (EGCG) has been reported to inhibit alcohol induced toxicity in HepG2 cells expressing CYP2E<sup>82</sup>.

### Resveratrol

A phytoalexin found in grapes and red wine, resveratrol exhibits anti-inflammatory and anti-oxidant effects. It has protective role against acute alcoholic liver injuries. In a clinical trial, resveratrol is shown to attenuate increased oxidative stress at a dose of 5 g/kg b.w. for 5 weeks in dietary intake by modulating malondialdehyde, superoxide dismutase, glutathione peroxidase and catalase levels<sup>83</sup>. It is also reported to alleviate alcoholic fatty liver in mice; resveratrol treatment increases sirtuin1 (SIRT1) expression and stimulates AMP-activated protein kinases (AMPK) activity in liver of ethanol-fed mice. The pathway is associated with suppression of sterol regulatory element-binding protein 1 (SREBP-1) and activation of PPAR-coactivator 1 $\alpha$  (PGC-1 $\alpha$ ).

Also, resveratrol markedly increases circulating adiponectin levels and mRNA expression of hepatic adiponectin receptors (AdipoR1/R2) that reduce lipid synthesis and increase fatty acid oxidation to prevent alcoholic liver steatosis<sup>84</sup>. In addition, it is reported to have protective effect against non-ALD through AMPK pathway<sup>85</sup>. Moreover, grape leaf extract (100 mg/kg b.w.) possesses antioxidant activity that can significantly reduce the levels of lipid peroxidation and restore the enzymatic and non-enzymatic antioxidants levels in alcoholic liver<sup>86</sup>.

### Quercetin

Quercetin also has been reported to possess protective activity against alcoholic hepatocyte injury. Quercetin treatment decreases lipid peroxidation and increases glutathione levels preventing alcoholic hepatotoxicity<sup>87</sup>. In addition, quercetin (10-200  $\mu$ M) protects human hepatocytes from ethanol-induced oxidative stress by heme oxygenase (HO)-1 upregulation. It activates MAPK signaling pathways through p38 and ERK mediated Nrf2 translocation and subsequent induction of HO-1 activity<sup>88</sup>.

Preventive treatment with quercetin significantly reduces IL-1 $\beta$ , IL-1, IL-6, IL-8 and TNF- $\alpha$  levels, whereas GSH and IL-10 levels are increased<sup>89</sup>. In addition, quercetin potentiates doxorubicin-mediated anti-tumor effect in liver through p53-mediated apoptotic pathways<sup>90</sup>. In another study, protective effect of quercetin, catechin and betaine have been observed against oxidative stress induced by ethanol *in vitro*<sup>91</sup>.

### Silymarin

Silymarin, a flavonolignan extracted from the milk thistle seeds, has been reported to counteract

oxidative stress in alcohol-induced liver injury. It is reported to decrease ethanol metabolism through inhibiting CYP2E1 pathway and ethanol-dependent cellular proliferation in hepatocarcinoma cell line<sup>92</sup>. In addition, silymarin pre-treatment at a dose of 60 mg/kg b.w. daily can reduce total collagen accumulation in liver even in severe fibrosis in rodent model<sup>93</sup>. It is also reported to retard the progression of alcohol-induced liver fibrosis in baboon model<sup>94</sup>.

### Lycopene

Lycopene, a major molecule in tomato, has antioxidant properties. Lycopene treatment (10  $\mu$ M) attenuates alcoholic apoptosis in HepG2 cells<sup>95</sup>. Although another study has reported that 3.3 mg/kg b.w. of lycopene treatment increases hepatic CYP2E1 protein and inflammation in alcohol-fed mouse<sup>96</sup>.

### Conclusion

The primary treatment for ALD is abstinence from alcohol. Along with nutritional factors, steroids, anti-cytokine and combined therapies are effective clinical practices, depending on the severity of the injury. Although most of the ALD patients return to alcoholism after a certain period of time, which further increases the severity of the disease and the risk of mortality. Moreover, malnutrition is very much associated in patients with severe ALDs. Standard nutritional therapy may improve liver function test and decrease the intensity of fatty liver formation. The beneficial effect of nutrition is effective in protective studies, indicating a probable reduction of complications, if nutritional supplementation is adequate while limited alcohol consumption is being continued.

Though several studies with different antioxidants, both enzymatic and non-enzymatic have been investigated on ALD, they are not very conclusive. Results on clinical trials for nutritional antioxidants are very few and still under investigation. Moreover, most antioxidant treatments are done in early stages of ALD and results are based on limited time period experiments. Some clinical trials with vitamin supplementation have reported faster recovery from acute alcoholic hepatitis, although vitamin restitution has not shown any improvement in mortality rates. Moreover, relevance of these different antioxidant researches lies within the treatment options in humans for clinical benefit, where further research are needed in the context of antioxidant therapeutics in ALD.

On the other hand, targeting MMP/TIMP balances during primary liver injuries can also be an alternate

way to treat ALD patients undergoing nutritional and anti-cytokine therapies. Recent progresses have been made in understanding biochemical and structural aspects of MMPs and their molecular complexes with TIMPs. The design of potent and specific inhibitors for MMPs represents a challenge for gaining insights into the biological roles of MMPs and also a chance for the development of new therapeutics in ALD.

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