

Prospect of mitochondria-targeted antioxidants as hepatoprotective and anti-alcoholic liver disease agent

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Abstract

Oxidative stress initiates and facilitates the disruption of the structural integrity of hepatic mitochondria, which leads to steatosis, steatohepatitis, fibrosis, and cirrhosis. It is now evident that mitochondrial dysfunction could be responsible for alcoholic liver disease (ALD). The challenge in treating ALD has been the limited availability of hepatoprotective agents and the lack of highly efficient delivery systems. Recent studies have shown that mitochondria-targeted therapies could address mitochondrial dysfunction (MD), which may greatly improve hepatoprotection and ALD treatment. This mini review discusses the potential role of mitochondria targeted antioxidants (MTAs) in the maintenance of hepatocellular integrity. This report also considers the mechanism of liver injury induced by alcohol and the progression of ALD from mitochondrial oxidative damage perspective as well as the possible mechanistic actions of hepatoprotective antioxidants. Preliminary studies suggest the prospect of MTAs as anti-ALD and hepatoprotective agents.

Key Words: antioxidants, alcohol metabolism, hepatoprotective, anti-ALD, mitochondrial dysfunction, mitochondria-targeted, oxidative stress, and cytochrome P450 2E1

Background

Long-term abuse of ethanol has culminated in worldwide increase in the morbidity and mortality of alcoholic liver disease (ALD). Pathologically, ALD progressively developed from steatosis (simple fatty liver) through steatohepatitis (inflamed fatty liver) to fibrosis and consequently cancer of the liver [1–3]. An increased incidence of ALD was evidenced in 2004 as 3.8% of all deaths worldwide were attributed to ethanol-induced chronic liver disease [4]. Moreover, an estimated 15% of known alcoholic cirrhosis patients develop hepatocellular carcinoma [5]. Environmental, genetic and nutritional factors have been identified to synergistically pathoprogress ALD to more acute disease [6]. Among these predisposing factors is oxidative stress, which is mediated by free radicals wherein has been reported to cause mitochondrial dysfunction (MD) and concomitantly fuel the pathogenesis of alcohol-induced liver injury [1]. The integrity of cells such as hepatocytes can be maintained by mitochondria which is considered as one of the significant functions of the organelle [7]. Also, liver cells have developed several mechanistic strategies which seek to forestall mitochondrial lesions effects namely increased mitochondrial biogenesis, disposal of damaged mitochondria induced by autophagy and signaling pathways regulation to assure energy metabolism [8]. Thus, functional mitochondria may reduce the impact of alcohol-evoked oxidative stress on hepatic injury.

Mitochondria is responsible for balancing the survival and death of hepatic cell through the activation of the intrinsic apoptotic pathway, while contributing to necrotic cell

death [8]. But the accumulation of damaged mitochondria could shift the balance between generation and scavenging of oxygen species (ROS) because of the overproduction of the species [9]. Therefore, severe alcohol-induced mitochondrial dysregulation could lead to hepatic cytolysis and its concomitant inflammation. To solve this health menace, engineered mitochondria-targeted antioxidants may be used to enhance oxidative homeostasis and limit liver cell death in view of the susceptibility of mitochondria to oxidative damage.

The search for effective drugs that are capable of protecting mitochondria against oxidative-induced damage is still ongoing. Natural protective mechanism of mitochondria is via interaction of several antioxidant systems [8], however, under stressed situations like ALD, this system is significantly overwhelmed [9,10]. Conventional antioxidants can be used to protect mitochondria but they have limited efficacy because of the difficulty in penetrating the mitochondrial membrane. Recent development of mitochondria-targeted ubiquinone, MitoQ₁₀ [11], has therefore instilled the hope of overcoming the problems of conventional antioxidants. MitoQ₁₀ can move through the phospholipid bilayer via its lipophilic triphenylphosphonium cationic group that is covalently attached to the ubiquinol antioxidant [12]. Thus, through drug delivery nanotechnology, the mitochondrial bioavailability of antioxidants can be improved. However, several studies should be done to comprehensively identify better strategies to deliver various natural antioxidants to hepatic mitochondria and all other human cells. Moreover, there is the need for further investigations to be conducted to affirm the initial reports of mitochondria-targeted antioxidants (MTAs) as an effective hepatoprotective and anti-ALD agent.

Reactive species generated from alcohol metabolism

Mitochondria strategically perform important metabolic and physiologic processes which are regulated in the cell, viz., production of adenosine triphosphate (ATP) [13], modulation and homeostasis of calcium [14], metabolism of nitrogen and amino acid [15], cell death due to apoptosis [16] and generation and detoxification of ROS [17,18] as well as biosynthesis of haeme and iron–sulphur centre [19].

Alcohol induced liver injury (AILI, acute and chronic) is displayed by morphological and functional modifications of mitochondria [5]. Since early 1960's, free radicals have been suspected to play active part in the progress of ethanol induced hepatic damage [20]. This because during ethanol metabolism in mitochondria and microsomes there is a direct generation of ROS and species of reactive nitrogen (RNS), which serve as a linkage between oxidative stress and dysfunction of mitochondria [21].

Actually, sustained concentrations of alcohol in the blood over a period of time determine its effects on diverse tissues. There is fast absorption of ethanol in the digestive tract via diffusion (passive manner) into walls of the stomach (approximately 20%), while the absorption of the other 80% occurs at the wall of small intestines, albeit 95-98% being metabolized wholly in the liver with few of it lost unaltered in sweat (0.10%), urine (0.30%) and breath (0.70%) [22–25]. The metabolism of ethanol in the human body involves three pathways including catalase, alcohol dehydrogenase (ADH) and microsomal ethanol-oxidation system (MEOS). Nevertheless, ethanol is mainly metabolized in human via the alcohol dehydrogenase pathway, which generates acetaldehyde and concomitant free

radical formation which alters levels of NADH and ratio of NADH/NAD⁺ redox [26,27].

Another system that participates in the oxidation of ethanol is electron transport in the microsomes which employs cytochrome p450 (CYP) enzymes to speed up the rate of reaction [28]. Generally, this pathway occurs in the hepatic smooth-endoplasmic reticulum of chronic alcohol consumers with the aim of eliminating toxic substances from the body through CYP2E1, however the 2E1 isoform significantly produces ROS and RNS species [29,30]. Besides, NADPH oxidases family, peroxisomes, xanthine oxidoreductases, lipoxygenases and cyclooxygenases have been identified as the other sources of ROS and RNS [31].

Available data suggest that high amounts of ROS, viz., the anionic superoxides ($\cdot\text{OH}$, H_2O_2 and $\text{O}_2^{\cdot-}$) are produced by an enhanced activity of NADPH oxidase of CYP2E1 [32,33]. The catabolism of ethanol also elevates the levels of mitochondrial reducing equivalents (i.e., NADH) which induces intermediates of semi-quinone (active redox state) that resides in complex (I and III) to become reduced, wherein O_2 is reduced to $\text{O}_2^{\cdot-}$ [34]. In mitochondria, the superoxide dismutase (SOD) like manganese superoxide dismutase (MnSOD) can convert $\text{O}_2^{\cdot-}$ to H_2O_2 [35], amidst possible and full reduction to H_2O or partial reduction to OH^{\cdot} (one of the powerful natural oxidants). Usually, reduced transition metals catalyze of OH^{\cdot} synthesis, which in sequence may be re-reduced by $\text{O}_2^{\cdot-}$, thereby proliferating this oxidative process [36]. During chronic alcoholism, there is increased generation of nitric oxide (NO^{\cdot}) via inducible NO synthase (iNOS) with the former quickly reacting with $\text{O}_2^{\cdot-}$ albeit the reaction being regulated by diffusion rate of the two radicals [37]. The NO^{\cdot} coupled with the peroxynitrite (ONOO^-) product formed are regarded as

principal mediators of alcohol-induced dysfunction of mitochondria [38]. Through post-translational modifications, proteins of mitochondria are inactivated by indirect or direct participation of ONOO^- in damaging reactions [39,40].

An unavoidable leakage of electrons from mitochondrial transport chain to molecular oxygen has been implicated as a significant site for the production of ROS [41]. In ALD, an increased in the leakage of electrons to oxygen is observed at I and III complexes, which results in alcohol-induced generation of $\text{O}_2^{\bullet-}$ (the major mitochondrial ROS, Fig. 1).

Mechanism of hepatic injury induced by alcohol

Existing data have affirmed alcohol as one of the principal cause of hepatic mitochondrial abnormalities that underlies ALD [1,42,43] and acute liver injury. Importantly, dysfunction of mitochondria is considered as the mechanism behind diseases and health conditions that occur in the liver and other organs such as the brain, muscle and heart. This may probably be due to the fact that these tissues require a lot of aerobic metabolism and thus they are more dependent on their mitochondria. Ninety percent (90%) of endogenous ROS are produced by mitochondria which have a greater cellular injury capacity [44]. As stated earlier, I and III complexes being the principal sites for generation of ethanol-induced $\text{O}_2^{\bullet-}$, OH^\bullet and peroxy radicals [45] can injure mitochondrial biomolecules such as DNA, lipids and proteins because of their high reactivity [46]. Especially, a defective complex I or III may increase reduction of O_2 to form $\text{O}_2^{\bullet-}$, which causes unserviceable damage to mitochondrial DNA [47–49].

Consequently, the ramification of ethanol-induced generation of ROS and RNS coupled with lower cellular antioxidant levels and oxidative stress is mitochondrial dysfunction [50], which results in hyperlipidemia with upregulation of adipose differentiation-related protein (ADRP) [51] and progression of hepatic injury [52]. Damage to other important complex cellular molecules like lipids and proteins may also occur during long-term exposure to alcohol.

The strong impact of oxidative stress induced by alcohol on mitochondria is due to the close proximity of the latter to the increased concentration of ROS and RNS. This initiates a vicious cycle whereby mutations in mtDNA compromise mitochondrial function, slowing down ETC/oxidative phosphorylation leading to high ROS/RNS levels, oxidative stress and mutations [44]. This is evident in mtDNA deletions (single or multiple) which has been reported to be prevalent and recurrent in alcoholic livers in comparison with controls (age-matched) [53]. The mtDNA modifications that characterize chronic ethanol treatment have been described to lower ETC encoded subunits in mitochondria, thereby culminating in damage of alcohol-induced respiratory activity in liver [54]. Actually, membrane potential (MMP) collapse in mitochondria via opening of the hepatic mitochondrial permeability transition pore (MPTP) is knowingly caused by oxidative mitochondrial damage [1]. Ethanol-induced oxidative stress encourages hepatic MPTP via Bax translocation to the mitochondria [55], however, opening hepatic MPTP contributes to mitochondrial swelling as well as precipitates hepatocyte necrosis and triggers apoptosis via cytochrome c release (Fig. 1) [56,57]. Thus, the contribution of oxidative mitochondrial damage to hepatic injury cannot be underestimated.

Apart from alcohol-induced oxidative stress causing mitochondrial dysfunction, it can also activate transcription factors (in the nucleus) that play a regulatory role in the expression of hepatic gene involved in the oxidation of fatty acid and response to inflammation [58].

The mechanistic interactions between chronic alcohol exposure, mitochondria, oxidative damage and inflammatory response could be found in work wherein a model was established by given alcohol to the subjects via intragastric infusion [59] (Fig. 1). Consequently, hepatic injury was induced by alcohol through the release of macrophage inflammatory protein-2 (MIP₂) and high cytotoxic cytokines levels from small Kupffer cells. More importantly, oxidative stress in mitochondria and its associated effects such as formation of carbonyl protein, pronounced peroxidation of lipids, production of lipidic and 1-hydroxyl ethyl radicals as well as reduced levels of endogenous antioxidant in liver (glutathione) may have accounted for the aforementioned phenomenon [60,61]. In addition, ALD hallmark may be due to the hepatic macrophageal sensitization to portal endotoxin or lipopolysaccharide (LPS) induced by alcohol [62]. Previous reviews have highlighted the pivotal role of LPS-induced signaling in initiating and progressing hepatic injury induced by alcohol [62]. Several lines of investigation have unearthed the interactions between inflammatory cytokines and ethanol-induced hepatic injury. These include the recognition of macrophageal Toll-like receptor 4 (TLR4) by LPS and other hepatic cell types as well as downstream activation of signaling pathways which culminate in NF κ B and activator protein-1 (AP-1) activation [63,64]. Furthermore, liver injury through prolonged alcohol consumption could be ascribed to LPS-induced extracellular signal-

regulated kinases (ERK) of the mitogen activated protein kinase (MAP) kinases family [65].

Sirtuin 1 (a nicotinamide adenine-dinucleotide, NAD⁺-dependent deacetylase, SIRT1) has been shown to control the activity of histones [66] and non-histone [67] proteins, namely peroxisome proliferator activated receptor- γ co-activator 1 α (PGC-1 α), which regulate synthesis of glucose and metabolism of energy [68]. The regulation of the pathways of gluconeogenesis and glycolysis by SIRT1 occurs in the liver via PGC-1 α [68], which in turn controls various reactions involving generation and utilisation of energy (for biogenesis of mitochondria, peripheral tissues uptake of glucose and thermogenesis) necessitating hepatic PGC- 1 α [69]. However, alcohol is postulated to reduce expressions of PGC- 1 α and SIRT1, whose interactions have been described to aid humans to adapt to deprivation of food and other metabolically induced disturbances [70]. Notably, alcohol-induced reduction of SIRT1 and PGC-1 α in mice could lead to mitochondrial dysfunction and subsequent liver injury. Thus, understanding the exact mechanism of liver injury/damage is very significant in identifying prominent lead antioxidants for ALD treatment. Consequently, therapies targeting basic processes of mitochondria viz., production of free radicals, or transcriptional factors activations, and specific interactions of proteins linked to diseases with mitochondria hold high prospects.

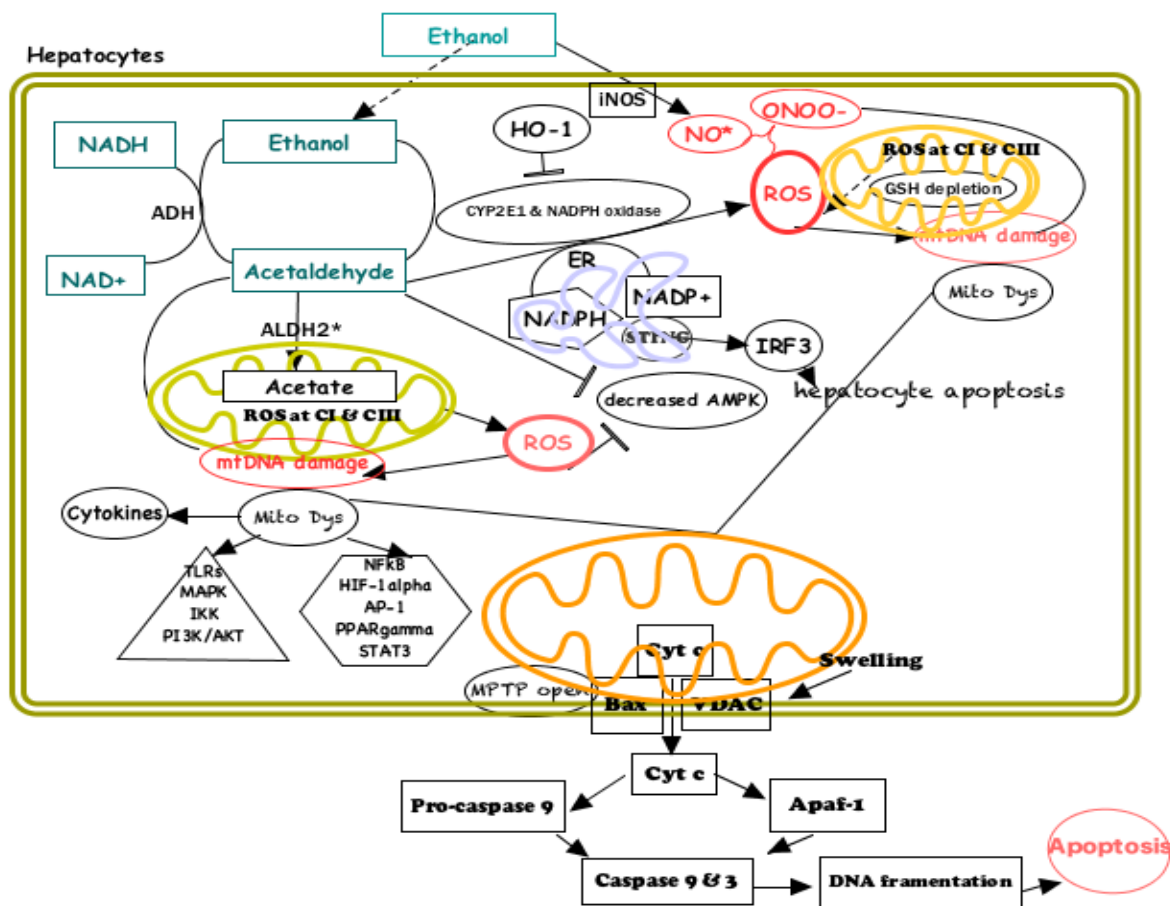


Fig.1 Schematic diagram showing the proposed molecular mechanism of mitochondrial dysfunction and hepatic injury induced by ethanol. Pathophysiology of liver injury induced by ethanol is considered as complex processes that involve the oxidative stress-induced mitochondrial dysfunction in hepatocytes which triggers an inflammation response as well as liver apoptosis. The stimulator of interferon genes, on the other hand, can encourage interferon regulatory factor 3 (IRF3) to induce hepatocyte apoptosis. AMPK: Adenosine 5'-monophosphate (AMP) activated protein kinase, AP-1: Activator protein-1, Apaf-1: Apoptotic protease activating factor-1, ALDH2^{*}: mitochondrial aldehyde dehydrogenase (inactive), Bax ((B-cell lymphoma 2 (Bcl2)-associated X protein)), CYP2E1: cytochrome P450 2E1, Cyt c: Cytochrome c release, DCs:

Dendritic cells, ER: Endoplasmic reticulum, GSH: Glutathione (reduced form), HIF-1 α : hsps: heat shock proteins, HO-1: Heme-oxygenase-1, IKK: I κ B kinase, Mito Dys: Mitochondrial dysfunction, NADH/NAD: Nicotinamide adenine dinucleotide (reduced and oxidized form), NADPH/NADP: Nicotinamide adenine dinucleotide phosphate (reduced and oxidized form), NF- κ B: Nuclear factor kappa B, PI3K/Akt: Phosphoinositide-3 kinase, PPAR γ : Peroxisome proliferator activated receptor γ , ROS: Reactive oxygen species, SAT3: Signal transducer and activator of transcription-3, STING: Stimulator of interferon genes, TLRs: Toll-like receptors, UPR: Unfolded protein response, VDAC: Voltage-dependent anion channel (VDAC).

Mechanistic action of hepatoprotective antioxidants

A preponderance of evidence put liver as the most significant site of xenobiotic metabolism, which in turn can cause its damage thereby leading to a deleterious functional effect. Hepatic mitochondria are an arbiter of cellular life and death by respiratorily and energetically controlling multiple pathways of signal transduction (intracellular and extracellular which have the tendency to cause mitochondrial dysfunction) and concomitant liver injury/death induced by apoptosis and necrosis [71,72]. To attenuate the harmful effects of oxidative damage, mitochondria contain inherent antioxidative systems of defense that are controlled tightly. They are capable of working in synergism to interrupt RNS/ROS activity, thus alterations in any of these antioxidative defenses may culminate in expansive and oxidative injury of mitochondria [73].

Antioxidants are compounds or systems that have the ability to counteract free radicals' damaging effect by direct inhibition of oxidation via triplet oxygen quenching and iron chelation [74] (Fig. 2). Enzymatic and nonenzymatic systems are the two principal kinds of hepatic mitochondrial antioxidant defense irrespective of whether they are endogenous or obtained from diets. Upon nuclear genome transportation to mitochondria, it encodes antioxidative enzymes such as thioredoxin reductase (TPx), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), [73]. Consequently, SOD catalyses the formation of H_2O_2 from oxygen and hydrogen, which is regarded as a significant endogenous antioxidant (enzymatic in nature) occurring in hepatic mitochondria. In mammals, 3 SOD isoenzymes (dependent on type of metal cofactor and protein folding) exist with the SOD1 consisting of SOD that binds to Cu-Zn and is a protein found in the cytoplasm and mitochondrion [75]. Besides, the mitochondrial matrix that expresses SOD2 (Mn-SOD) has manganese ion as its cofactor, while the secretion of SOD3 (EC SOD) into extracellular space took place after it has been translated into protein [76], albeit the enzyme's activity being increased after chronic alcohol feeding in mice [77]. Moreover, GPx mostly detoxifies peroxides of lipid and H_2O_2 using cofactor (reduced form of glutathione, GSH). Usually, the oxidised form GSH (glutathione disulfide, GSSG) after the detoxification process. The good news is that with NADPH as an electron donor, glutathione reductase can regenerate GSH. Furthermore, GSH scavenges singlet oxygen (1O_2) and OH radical directly, while maintaining the reduced forms of vitamins C and E, thereby acting as a nonenzymatic antioxidant. In addition, the oxidant property of mitochondrial aldehyde dehydrogenase 2 (ALDH2, a principal enzyme in ethanol metabolism) has been established, especially in the prevention of ROS generation induced

by ethanol or aldehyde [78,79]. Nevertheless, its enzymatic activity has been reported to be compromised via direct deacetylation by sirtuin 3 (SIRT3) [80].

Oxidative damage through ROS/RNS generation has been implicated as significant mechanisms involved in the hepatotoxic effect of alcohol. In this regard, antioxidants can therefore be applied to protect liver by lowering alterations in hepatocellular nucleic acids, proteins and lipids induced by ethanol [81]. In general, the sacrificial or preventive roles through which cellular biomolecules in liver are protected by antioxidants include metal ions chelation to decompose peroxides of lipid or avoid reactive species production, O_2^- quenching to prevent peroxides formation, ROS scavenging to limit initiation of peroxidation and localized reduction of O_2 concentrations [82]. Actually, sacrificial effect of antioxidant alludes to the capacity to block chain reactions of free radicals through scavenging activity but preventing role indicates the inhibition of reactive species formation [83]. Solubility, activation energy, rate constant, redox potential, stability and molecular structure as well peroxy accessibility to systems of micelles, membrane and emulsions have effect on the effectiveness and chemical potential of antioxidants [82,84].

Hepatoprotective antioxidants are either endogenous or exogenous, which are used to ameliorate pathological alterations induced by oxidative stress-related injury. Detailed mechanism of antioxidants in hepatoprotection is still not clear despite extensive work, notwithstanding, several mechanisms have been proposed. Thus, antioxidants seem to protect hepatocytes against injury induced by alcohol via attenuation of elevated levels of enzymes in serum lipid peroxidation, ROS/RNS generation, up-regulation and activation of endogenous antioxidants systems, as well as free radicals scavenging [85,86].

Kamoun and colleagues recently investigated the antihepatotoxic and antinephrotoxic effect of active ingredient of *Sardinella aurita* (Sardinelle) against oxidative stressed rats induced by alcohol [87]. The authors reported that lipid peroxidation inhibitory activity of Sardinelle was ascribable to the interaction of between its peptides structure and free radicals, thereby resulting in the protection of liver via antioxidation. Thus, the imidazole functional group of histidine in Sardinelle donated proton which aided the radical scavenging activity of the protein [87]. Possibly, this lead compound could quench the excessive oxidation of free radicals via the donation of hydrogen [88]. In addition, Kumar and workers [86] posited that lucidone-mediated upregulation of endogenous antioxidant haeme oxygenase 1 (HO-1, an inducible form) in hepatic hepG2 cells of human origin through Nrf-2 signaling pathway as the principal mechanism for its hepatoprotective action. Besides, HO-1 has been observed to play a vital role in homeostasis of iron and antioxidant defense in living cells [89]. This mechanism was evident in previous investigations which suggested that an increase in Nrf-2 activity strongly protected hepatocytes against oxidative stress induced by ethanol [90,91]. Moreover, cannabidiol was shown to exhibit its antihepatotoxic property against acute steatotic liver in mice induced by alcohol through antioxidation, viz., JNK MAPK pathway activation and increased oxidative stress prevention [92]. In an experimental investigation, the hepatoprotective potential of resveratrol was shown in oxidative liver damage through radicals scavenging and proinflammatory cytokines [93]. However, current data from clinical trials for ALD are lacking though human studies on preparations of essential phospholipids, silymarin or vitamin A seems popular previously.

Owing to the significance of liver playing the role of metabolizer, detoxifier and regenerator, more studies should be directed at developing multitarget hepatoprotective antioxidants. In this regard, understanding the mechanistic actions of an ideal hepatoprotector will not only become academically prudent, but may also lead to the development of highly specific hepatoprotective and anti-ALD drugs for clinical applications.

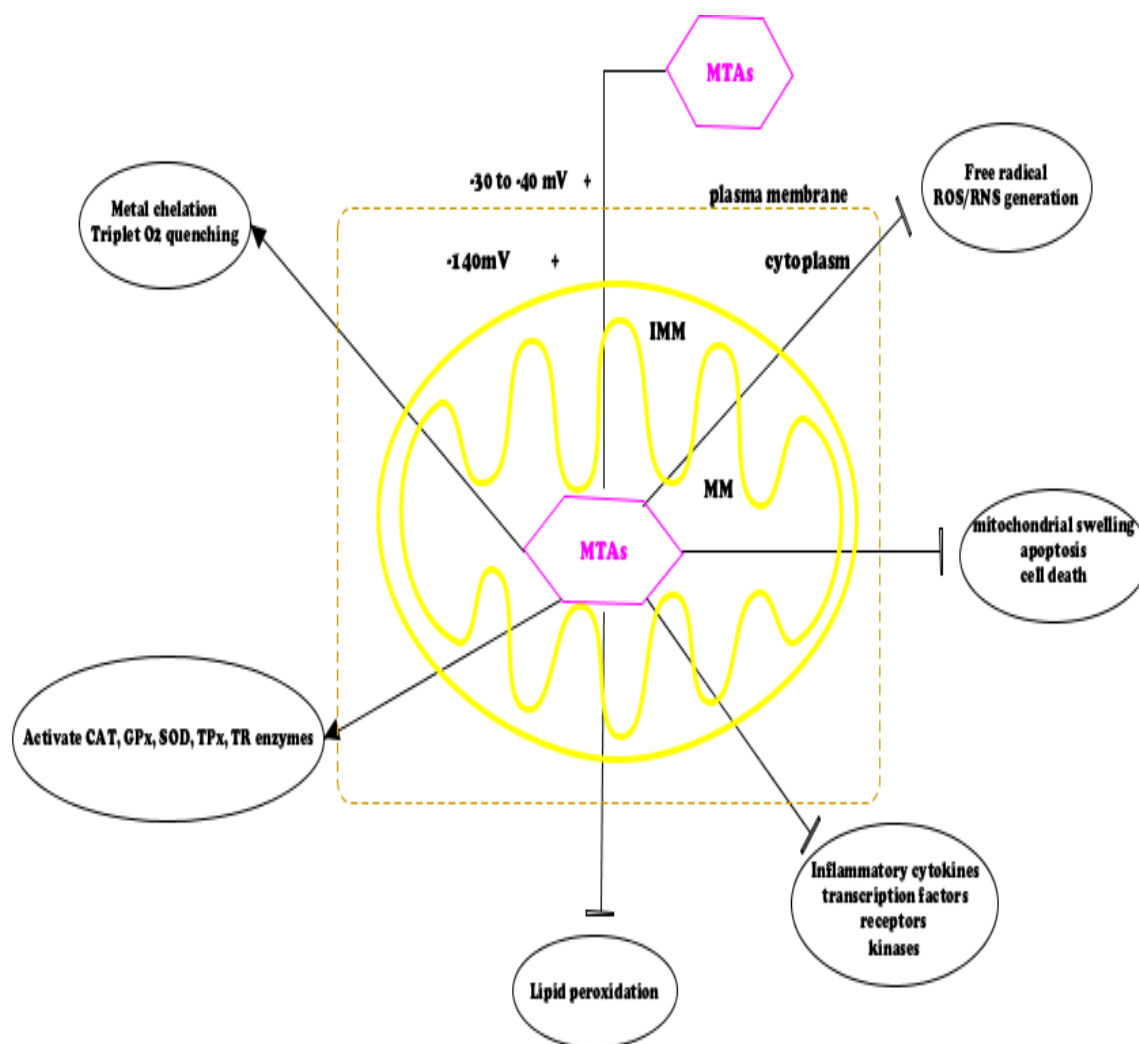


Fig. 2 Schematic diagram showing the proposed mechanistic of action of antioxidants against free radical-induced human damage. They carry out their hepatoprotective

functions by attenuating lipid peroxidation, reactive oxygen species/reactive nitrogen species (ROS/RNS) generation, up-regulation, reduction of calcium accumulation [94], and activation of endogenous antioxidants systems, as well as direct scavenging of free radicals. Catalase (CAT), Glutathione peroxidase (GPx), mitochondrial-targeted antioxidants (MTAs), superoxide dismutase (SOD), thioredoxin peroxidase (TPx) and thioredoxin reductase (TR),

Targeting antioxidants to mitochondria, future prospect for hepatoprotective and anti-ALD potentials

As stated earlier, available evidence has suggested the contribution of mitochondrial dysfunction and liver cell death in ALD [1,2]. In this regard, antioxidants have been increasingly explored for their potential therapeutic efficacy on oxidative-induced dysfunction in mitochondria. However, antioxidants concentration should essentially be raised in mitochondria where they are most required against the backdrop of the low bioavailability of conventional antioxidants to the mitochondria interior [95]. This can be achieved by targeting scavenging compounds to mitochondria to modulate the levels of ROS/RNS and their inducement processes, viz., permeability transition and cell death in mitochondria [96]. Already, MTAs have been therapeutically utilized in models of some pathological experiments. Notably, among them is antioxidant derivative targeted to mitochondria such as mitoquinone (MitoQ) which has ubiquinone as natural carrier of electrons in the respiratory chain in mitochondria. Indeed, a cation of lipophilic triphenyl-phosphonium (TPP⁺) is covalently conjugated to ubiquinone to form MitoQ which results

severalfold accumulation in cytosol and several hundredfold in mitochondria [97], wherein its antioxidant potential becomes evident.

Inner membrane potential of mitochondria has been estimated via derivatives of long-established phosphonium. Notably, the ability of TPP⁺ to quickly and directly permeate lipidic bilayers without losing their positive charges is advantageous to the above-mentioned process. The hydrophobic surface that surrounds positive phosphorus atom can facilitate the accumulation of TPP⁺ in the inner core of mitochondria. In particular, TPP⁺ accumulation in mitochondria inside is driven by the maintenance of electric potential difference across the inner membrane of mitochondria (induced by respiratory chain) coupled with the capacity of bulky hydrophobic cations to permeate membranes. Another novel way to target antioxidants to mitochondria using TPP⁺ is through nanoparticles development. Kwon and colleagues [98] successfully designed and synthesized nanoparticles loaded with TPP-conjugated ceria, which was localised to mitochondria and pharmacologically suppressed neuronal death in Alzheimer's disease modeled using a 5 familial AD-associated mutations (5XFAD) in transgenic mice. Also, through derivatives of chitosan, Chen and co-workers [99] also designed and prepared novel multifunctional nanoparticles targeting mitochondria (MNPs) for the delivery of anticancer drugs to liver.

Ubiquinone is the same antioxidant constituent in MitoQ which is found in coenzyme Q10 [97]. In mitochondrial respiratory chain, complex II quickly activate ubiquinone in MitoQ to active ubiquinol (antioxidant form) [100]. Usually, after detoxification of ROS, ubiquinone is re-formed from ubiquinol in MitoQ and vice versa [100]. Through this technique, MitoQ can act as an important antioxidant for mitochondrial

targeting.

Based on MitoQ model, Chacko and co-experimenters [101] demonstrated the ability of MitoQ to alleviate liver steatosis induced by alcohol in mitochondria in groups that were fed ethanol. Besides, the authors observed that MitoQ was able to attenuate various negative effects of mitochondrial-linked ROS/RNS, such as inhibition of protein nitration and formation of protein aldehydes as well as stabilization of ROS-dependent (hypoxia-inducible factor 1alpha (HIF-1 α) [101]. To support this finding, another work reported that MitoTEMPOL (mitoT) could ameliorate oxidative stress-associated dysfunction of mitochondria and alveolar macrophages in mice induced by alcohol, albeit no data to support hepatoprotective activity of mitoT [102].

However, the challenge with TPP⁺ derivatives is that the rate and extent of their accumulation is affected by their hydrophobicity, while, in comparison with hydrophilic derivatives, the lipophilic TPP⁺ quickly accumulated in mitochondrial at higher concentrations [103,104]. Again, Trnka and co-workers [105] postulated that various derivatives of hydrophobic TPP⁺ can negatively affect membrane potential of mitochondria and the activity of respiratory chain together with the induction of mitochondrial proton leakage. To overcome these limitations, the search for a more natural MTAs should be intensified, as they have the prospect for achieving maximum hepatoprotective efficacy against alcohol-induced liver damage with minimum side effects to mitochondrial locality and liver cells in general. Also, Zhang and colleagues [106] evaluated the hepatoprotective and anti-ALD activities of demethyleneberberine (DMB), MTA that occur naturally in *Cortex Phellodendri chinensis* (Chinese herb). After ethanol administration (both acute and

chronic) to mice and *in vitro* study in HepG2 cells, the authors observed that DMB ameliorated mitochondrial dysfunction caused by acutely induced oxidative stress [106]. The authors also identified that DMB was able to accumulate in hepatic mitochondria due to its chemical structure while protecting the liver against harmful effect of ethanol through the suppression of HIF-1 α , iNOS synthase and CYP2E1 [106].

The few available data on hepatoprotective and anti-ALD activities of MTAs look promising. However, more preclinical investigations into efficacy of MTAs against ALD should be conducted using clinical trials. Preferably, to satisfy the unmet need of ALD therapy, natural antioxidants that are capable of ameliorating mitochondrial oxidative damage, modulating inflammatory, restoring mitochondrial biogenesis and liver regeneration can be designed and delivered using novel mitochondrially targeted nanomaterials

Conclusion

Hepatic mitochondrial oxidative damage is clearly pivotal in the pathological process of liver injury induced by alcohol. Anatomically, ALD encompasses a variety of liver cells, which makes drug delivery into this organ complicated. Although absolute abstinence is a significant therapeutic precaution, few experimental data on MTA (as treatment options for ALD) have shown promising outcomes. Nevertheless, the mitochondrial-targeted model will pave way for the establishment of hepatoprotective and anti-ALD drugs. Hence, more novel and innovative works are desired to thoroughly unearth more natural MTAs as hepatoprotective and anti-ALD agents.

Authors' contributions

MAF and JA conceived the concept, collected information, discussed issues and wrote the manuscript. JA discussed the issues. The final manuscript was read and approved by the authors.

Competing interests

No potential conflicts of interest

Consent for publication

Manuscript was approved for publication by both authors.

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