Alcoholic liver diseases: Disease spectrum, pathophysiology, oxidative stress and role of flavonoids in alcoholic liver injury

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Article History:
Received on: 07 Dec 2019
Revised on: 01 Jan 2020
Accepted on: 02 Jan 2020

Keywords:
Alcoholic liver diseases, disease spectrum, oxidative stress, flavonoids, Pathophysiology

ABSTRACT
An Alcoholic Liver Disease (ALD) has emerged as the serious health problem globally, with high morbidity and mortality rate. Heavy alcohol consumption resulted in broad spectrum of liver diseases ranging from steatosis (alcoholic fatty liver), alcoholic hepatitis (AH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). In this review the detailed pathophysiology of the ALD, role of oxidative stress ALD, the mechanism of action of flavonoids with different models used have been explained. Heavy alcohol consumption leads to severe liver diseases, which is demonstrated by increased blood levels of ALT, AST, and/or lactate dehydrogenase (LDH) and accumulation of lipid in the hepatocytes causing steatosis. There are three mechanisms involved in causing of ALD, those are: (a) acetaldehyde toxicity; (b) oxidative stress or generation of ROS; and lastly (c) provocation of the immune response of hepatocytes causing oxidative stress. This shows that oxidative stress is a major cause for ALD, therefore, defence activities against this stress are most vital in the prevention of ALD. Application of antioxidants especially flavonoids signifies a rational curative strategy to prevent and cure liver diseases involving oxidative stress. Flavonoids inhibit ROS formation by regulating glutathione S-transferase, mitochondrial succinoxidase etc. Since lipid peroxidation is a result of oxidative stress, lipids are protected from oxidative damage using flavonoids. Various flavonoids and their mechanism of action have been discussed in this review, formulation of these flavonoids will be highly beneficial for treatment of ALD.

INTRODUCTION
Alcoholic Liver Diseases (ALD) are the major ubiquitous liver diseases in countries like Europe and USA (Pimpin, 2018). Alcohol consumption globally kills around 3.3 million human populations per annum, which accounts for 5.9% of all deaths (Ohashi et al., 2018). It has been well-documented that alcohol consumption is accountable for the progress of a disease condition itself or obliquely contributes to the development of other liver diseases.
Table 1: Representing the list of flavonoids, their mechanism of action, the model used and outcomes of the study in alcoholic liver diseases.

| SL NO | Flavonoid                  | MOA                                      | Model                        | Outcome/Results                                                                 | References                                                                 |
|-------|----------------------------|------------------------------------------|-------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| 1     | Apigenin (4', 5, 7-trihydroxyflavone) | ↓CYP2E1 protein ↓NF-kB protein ↓IkB-a protein. | Rat BRL hepatocytes          | Inhibit oxidative stress and LPS-induced inflammation further treating ALD.     | (Wang, 2018, 2017; Guo, 2017; Lu, 2016; Zhou, 2017; Lu, 2018; Zhou, 2018a; Lin, 2017; Zhou, 2018b; Jiang, 2017; Li, 2018; Liu, 2018; Lee, 2018; Szkudelska et al., 2017; Ma, 2017). |
| 2     | Apigenin (4', 5, 7-trihydroxyflavone) | ↓CYP2E1 ↓SREBP-1c, ↓DGAT, ↓NFkB          | Kunming mice                 | It protects ALD by inhibiting of CYP2E1 mediated ROS generation, and regulation of PPAR-α mediated lipogenic and inflammatory gene expression. Attenuate fatty acids synthesis and bio-synthesis of unsaturated fatty acids thus prevents alcoholic fatty liver. Inhibit hepatocyte necroptosis thus may be used in the treatment of ALD. |                                                                         |
| 3     | Curcumin                   | ↓fatty acid synthase ↓pyruvate metabolism | Kunming mice                 |                                                                                 |                                                                         |
| 4     | Curcumin                   | It modulates of Nrf2/p53 pathway         | In Vitro - Human hepatocyte LO2 cells In Vivo - mice | In Vivo and In Vitro - It potently improved alcohol-induced fat deposition providing effective treatment of ALD. |                                                                         |
| 5     | Curcumin                   | IN VITRO model revealed that it ↓Nrf2 ↓FXR | In Vivo - Human hepatocyte LO2 hepatocyte cells In Vivo- Sprague-Dawley rats |                                                                                 |                                                                         |
| 6     | Hesperidin                 | It reduces ER stress and damage DNA, by regulating alcohol and fat metabolism. | Wild-type - AB strain zebrafish | It inhibited Hepatic steatosis and injury in zebra fish induced by alcohol       |                                                                         |
| 7     | Isorhamnetin               | Apoptosis and autophagy via The p38/ppar-α pathway was inhibited in mice. | Male Balb/C mice |
| 8     | Kaempferol                 | Inhibits SP1, Hsp70 and CYP2E1           | Mouse primary hepatocytes mice model | It contributed to hepatoprotection in ALD                                        |                                                                         |
| 9     | Kaempferol                 | ↓CYP2E1                                   |                               | It decreased the oxidative stress as well as lipid peroxidation and show hepatoprotective activity against alcohol-induced liver injury |                                                                         |

Continued on next page
| SL NO | Flavonoid     | MOA                        | Model               | Outcome/Results                                                                                                                                                                                                 | References          |
|-------|---------------|----------------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| 10    | Luteolin      | ↓phosphorylation of SREBP-1c| mouse AML-12 hepatocyte cells | It is effective in the treatment of alcohol-induced steatosis.                                                                                                                                                     |                     |
| 11    | Naringin      | It reduced alcoholic liver morphological phenotypes | Wild type zebrafish. | It inhibited alcohol-induced liver steatosis and injury.                                                                                                                                                            |                     |
| 12    | Naringin      | It diminishes lipid accumulation and decreasing oxidative stress and apoptosis | Wild-type (wt) zebrafish. | It may inhibit ALD and steatosis.                                                                                                                                                                                  |                     |
| 13    | Oroxylin A    | It decreased the nuclear translocation of HIF-1α | Human LO2 hepatocyte cells | It inhibits lipid accumulation and regulated the genes of hepatic lipid metabolism thus improved alcoholic liver injury.                                                                                           |                     |
| 14    | Oroxylin A    | ↓p16, p21, and Hmga1, ↓Yes-associated protein (YAP) | In Vivo- Male ICR mice | It inhibits of ethanol-induced hepatocyte senescence and leads to improvement of alcoholic hepatocellular injury.                                                                                                  |                     |
| 15    | Oligomeric proanthocyanidin (OPC) | It inhibits of HSC activation suppressing NF-kB activation through JNK/ERK, MAPK and PI3K/Akt phosphorylation | In Vivo- Male ICR mice, In Vitro - Human hepatocyte cell line LO2 HSC-T6 -cell line. | It acts by anti-fibrotic mechanism and treats of alcoholic liver fibrosis.                                                                                                                                   |                     |
| 16    | Oligomeric proanthocyanidin (OPC) | ↓ALT,AST, TG, TC, LDL-c and MDA, ↑HDL-c and SOD, ↓expressions of Srebp-1c, Srebp2, IL-1b, IL-6 and TNF-a. | In Vivo- Female mice C57BL/6 | It improved alcohol-induced dyslipidemia and alleviated liver steatosis                                                                                                                                               |                     |
| 17    | Quercetin     | ↓LC3-II and p62 accumulation, ↑expression of LAMP1, LAMP2, and Rab7 | C57BL/6J mice | It acts by ameliorating lysosomal autophagy dysfunction and can be used for prevention of ALD.                                                                                                                   |                     |

*Continued on next page*
| SL NO | Flavonoid                  | MOA                                                                 | Model          | Outcome/Results                                                                 | References                                                                 |
|------|---------------------------|----------------------------------------------------------------------|----------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| 18   | Quercetin                 | ↑expression Nrf2, ↓ROS/NF-κB/NLRP3, IL-1β, IL-18 pathways by indising heme oxygenase-1 | Male SPF-Wistar rats | It prevents inflammation, oxidative stress in ALD and showed promising treatment target for AALI. |                                                                           |
| 19   | Quercetin and its metabolites | ↓ROS generation, ↑the reduced form of GSH, ↑SOD or CAT, ↑HO-1 expression activates Nrf2 and AP-1 | HepG2 human hepatocarcinoma cell line | Diminishes lipid Peroxidation, have cytoprotective ability against ethanol-induced oxidative stress and for ameliorating alcohol-induced liver disease. |                                                                           |
| 20   | Resveratrol               | It reduced liver lipid content, probably due to a decrease in fatty acid synthesis. It also decreased also blood levels of triglycerides and free fatty acids and reduced γ-glutamyl transferase activity. | Wistar rats | It Alleviated Ethanol-Induced Hormonal and Metabolic Disturbances in the Rat.   |                                                                           |
| 21   | Resveratrol               | It inhibited the per-oxidation of lipids and improving the activity of antioxidant enzymes. It also showed the inhibitory production of nitric oxide and tumor necrosis factor α (TNF-α). | Wistar albino rats | It prevents the liver from ethanol-induced oxidative damage thus prevents ALD. |                                                                           |
| 22   | Resveratrol               | It reduced ROS production in activated platelets and inhibit HIF-1α expression. | Male Sprague-Dawley (SD) rats | It acts as a potential agent for the treatment of AFLD. |                                                                           |
Such injurious outcome is associated with alcohol consumption: (i) first of all, the amount of alcohol consumed i.e., dose-dependent relationship (the amount of alcohol intake varies globally but normal consumption is binge drinking and also chronic alcohol intake of more than 40 g/day); (ii) secondly, consumption pattern i.e., time-dependent consumption (e.g. heavy periodic drinking or chronic intake); and (iii) lastly, the type of the drink consumed (e.g. household or illegitimate alcoholic drink contaminated with extremely noxious material).

Liver is the major target site for alcohol-induced injury and chief site for alcohol/ethanol metabolism. Because of the higher strength of alcohol in the hepatic blood circulation along with the metabolic consequences of alcohol the liver is highly susceptible to alcohol-induced toxicity. ALD embraces a broad range of diseases emerging from increased alcohol consumption, as steatosis (also known as fatty liver) to alcoholic hepatitis, fibrosis, cirrhosis and finally causing Hepatocellular carcinoma (HCC).

Steatosis is developed when accumulation of liver fat (triglycerides) is observed due to heavy alcohol intake, accounts for more than 5 to 10 % of the liver mass (Seitz, 2017). Around 90% of heavy alcohol consumers develop steatosis, but only 35% of them develop an advanced form of ALD. This takes in consideration two major points: (i) these comprise mainly factors like sex, obesity, pattern of consumption and beverage type, dietary factors, non-sex-linked genetic factors, smoking, age, race/ethnicity and drugs; and (ii) there are chances that may interfere in the initial/reversible stages of the disease to avoid its development to more severe stages (Choi and Diehl, 2017). Alcoholic hepatitis (AH) is inflammation of the liver that is signalized by definite histological description i.e., fat, ballooning degeneration of hepatic cells containing Mallory-Dank bodies, infiltrating Neutrophils caused by chronic alcohol intake. Alcoholic fibrosis is characterized by the extreme deposition of extracellular matrix protein including collagen caused by excessive alcohol intake. Alcoholic cirrhosis is elucidated as chronic alcohol consumption responds to the histological development of regenerative nodules surrounded by fibrous bands. HCC is a primary tumor that develops most recurrently in liver cirrhosis (Seitz, 2017).

Disease Spectrum

Binge alcohol intake results in a broad range of hepatic lesions, ranging from simple alcoholic fatty liver (also known as alcoholic steatosis), alcoholic hepatitis (AH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (refer Figure 1 for histology of disease spectrum).

First response of ALD observed due to heavy and habitual alcohol intake is steatosis developed in more than 90% of heavy alcohol consumers. The early and mild type of steatosis in zone 3 (perivenular) hepatic cells; with more severity of liver damage which can also influence zone 2 and also zone 1 (periportal) hepatic cells. Interestingly, around 30% of profound alcohol consumers develop the most severe stages of liver injury, which includes alcoholic fibrosis and cirrhosis. In individuals with primary ALD and heavy drinkers, could develop the different episodes of superimposed AH. The AH leads to severe problems associated with liver injury, portal hypertension and has high short-term mortality. Probably around 80g of alcohol per day or equivalent to 6 to 8 normal drinks daily for many years can lead to a more advanced form of liver disease. The women have a considerably higher risk of developing alcoholic liver diseases than men for the above-given amount of alcohol intake.

The stages of ALD finally leading to HCC are briefly explained

Steatosis develops in alcoholics who consume 4-5 standard drinks/day for years. The hepatocytes in this state is distinguished by the fat deposition, observed microscopically as lipid droplets, initially surrounding the liver’s central vein (i.e., perivenular hepatocytes) in zone 3 (centrilobular zone), then progressing to mid-lobular hepatocytes i.e., zone 2, and ultimately to the hepatocytes that surround the hepatic portal vein (i.e., periportal hepatocytes) zone 1 (periportal zone). (Osna et al., 2017) Although steatosis is reversible by 4-6 weeks of abstinence (Ohashi et al., 2018), patients who continue drinking, steatosis is a risk factor for progression to fibrosis and cirrhosis. Among 20 to 40% of constant heavy drinkers develop AH, which is characterized by fever, and hepatomegaly in association to fibrosis and cirrhosis. Among 20 to 40% of constant heavy drinkers develop AH, which is characterized by fever, and hepatomegaly in association to the clinical and laboratory features of liver failure. Steatohepatitis is a more serious, inflammatory form of liver disease. The hepatocytes are distinguished by swollen, ballooning degeneration, neutrophilic infiltration, and the growth of tangled aggregates of insoluble proteins called Mallory-Denk bodies. Central to hepatitis development is the activation of KCs (Kupfer cells), the resident liver macrophages (Osna et al., 2017).

Fibrosis is characterized by the deposition of unusual amounts of extracellular matrix proteins (ECM), which are activated by human stellate cells (HSC). Individuals with ALD initially exhibit active pericellular fibrosis, which may progress to cirrhosis, the late stage of hepatic scarring (Osna et al., 2017). Cirrhosis is developed in heavy drinkers
Figure 1: Spectrum of alcoholic liver diseases

Figure 2: The development of steatosis contributed by hepatic and extrahepatic mechanisms

Figure 3: Depiction of Pathophysiology of Alcoholic fatty liver (steatosis)
compared to binge drinkers. It is characterized by collagen accumulation that can be pericellular, in the space of Disse, and around central veins (central hyaline sclerosis). Collagen develops a bridge between central veins and portal tracts isolating groups of hepatocytes which form the regeneration nodules (Choi and Diehl, 2017). Cirrhosis is a major risk factor for the progression to HCC, due to the consumption of a large volume of alcohol. The 10 years of cumulative frequency of HCC was observed from a range 6.8% to 28.7%.

Pathophysiology of ALD
In the 1960s, the biological effects of alcohol on the liver were revealed by human and rodent studies. Various enzymes present in the mitochondria of the liver metabolizes the alcohol like aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), and the microsomal ethanol oxidizing system (MEOS). Adak (2018). When the alcohol con-
concentration in the blood is lower, ADH oxidizes it to acetaldehyde, these lead to the formation of NADH and NAD and change the redox state of the cell. Whereas MEOS performs at moderate to a high level of alcohol. These changes the functional properties of the hepatic cell membrane leading to ALD.

**Alcoholic Fatty Liver (Steatosis)**

Histologically steatosis is defined and characterized by the accumulation of fat molecules in hepatocytes as micro vesicular and macro vesicular droplets leading to an increased intracytoplasmic triglyceride production, phospholipids, and cholesterol esters.

The hypothesis to explain this accumulation introduced the generation of redox shifts by aldehyde dehydrogenases on oxidation of alcohol in the hepatocytes, oxidative stress and the transfer of peripheral triglycerides from adipose tissue to the liver (Livero and Acco, 2016).

Acetaldehyde or alcohol does not directly contribute the fatty acid synthesis, but, acetate the metabolite of acetaldehyde, generated in mitochondria can be converted to acetyl-CoA, which is useful in fatty acid synthesis (Seitz, 2017).

The ratio of NADH/NAD+ in hepatocytes is elevated by alcohol consumption, which interrupts the mitochondrial beta-oxidation of fatty acids and leads to steatosis (Gao and Bataller, 2011).

### Figure 6: Category of flavonoids with examples.

| Category of Flavonoids | General Structure | EXAMPLES |
|------------------------|------------------|----------|
| **Flavones**           | ![Flavone Structure](image) | Luteolin, Apigenin |
| **Flavanones**         | ![Flavanone Structure](image) | Hesperetin, Naringenin |
| **Flavanonol**         | ![Flavanol Structure](image) | Taxifolin |
| **Flavanols**          | ![Flavanol Structure](image) | Quercetin, Kaempferol |
| **Isoflavones**        | ![Isoflavone Structure](image) | Genistein, Daidzein |
| **Flavan-3-ols**       | ![Flavan-3-ol Structure](image) | Catechin, Epicatechin |
On the other hand, alcohol-induced redox change by itself did not completely describe why the liver rapidly deposited fat. New current studies strongly supported the concept that alcohol-induced alcoholic fatty liver is multifactorial as elaborated below (refer Figure 2).

**Enhanced hepatic lipogenesis by alcohol**

Higher expression of lipogenic enzymes and cytokines lead to an increased lipogenesis which are encoded by genes regulated by two transcription factors, such as sterol regulatory element-binding protein-1c (SREBP-1c) and early growth response-1 (Egr-1).

Researchers showed that when rodents exposed to heavy alcohol feeding, alcohol intake decreases adipose tissue mass by increasing fat breakdown (i.e., lipolysis) in adipose tissue (Wang, 2016). This breakdown leads to the formation of free fatty acids, these are then deposited in the liver and esterified into triglycerides, therefore, accumulating the fat in the liver.

On consumption of alcohol, the formation of acetaldehyde may directly enhance the or otherwise it can indirectly increase SREBP-1c expression by process of activation and factors which will activate SREBP-1c expression, such as the endocannabinoids, adenosine, LPS signaling through Toll-like receptor-4 (TLR-4), endoplasmic reticulum response to cell stress, and it inhibits proteins like IRF-3, tumor necrosis factor (TNF)-alpha or Egr-1 (Zhao, 2019).

Alcohol consumption will also inhibit factors that decrease SREBP-1c expression, which includes AMP-activated protein kinase (AMPK), adiponectin, and a signal transducer, Sir2, (You, 2019) and activator of transcription 3 (STAT3). This disruption of SREBP-1c in mice model decreased steatosis, representing its function in ALD.

Alcohol can also influence the enzymatic activities involved in the metabolism of fat in the liver by inhibition of AMPK that reduces the metabolism of fat and steatosis. This inhibition leads to elevated ACC activity and reduced carnitine palmitoyltransferase 1 (CPT1L) activity each of which has a vital role in the steatosis development.

**Hepatic lipid degradation by alcohol**

Since many lipids (fats) in hepatic cells are deposited as lipid droplets, for their subsequent oxidation these cell organelles have to be primarily degraded to extract their lipids. This degradation of lipid droplets is carried out by a specific intracellular process known as lipophagy also called autophagy. During this process, lipid droplets are engulfed by autophagosomes (double membrane-bound vacuoles).

There are several reasons for the slowdown of β-oxidation: First of all, the increased generation of NADH by alcohol oxidation. Secondly, the generation of acetaldehyde deactivates the PPAR-α (peroxisome proliferator activated receptor alpha), that covalently binds to the transcription factor therefore, blocking its capacity to bind PPAR-α promoter sequences that act along with the retinoid X receptor (RXR), which regulates fatty-acid transport and oxidation. Thirdly, oxidation of alcohol leads to mitochondrial depolarization, by impairing their abilities to generate ATP and causes leakage of outer membranes of the hepatocytes, results in decreased fatty acid importation and β-oxidation. At the fourth, alcohol consumption decreases the production of the adiponectin a hormone that is secreted by (i.e., fat cells).

The acetaldehyde straight away suppresses the transcriptional activation activity and DNA-binding ability of PPAR-α in hepatocytes (refer Figure 3). (Gao and Bataller, 2011). Alcohol intake can also indirectly inhibit PPAR-α through up-regulation of CYP2E1 and adenosine, together inhibit PPAR-α, or through decreasing of adiponectin and zinc, that trigger PPAR-α.

**Alcoholic Hepatitis**

Alcoholic hepatitis is seen in 30–40% of patients with chronic alcohol intake. Hepatocytes are characterized by Ballooning degeneration including Mallory-Denk bodies, infiltrating neutrophils, and “chicken wire”–like pattern of fibrosis.

**Hepatotoxic effects of ethanol**

The metabolism of alcohol which is instrumental in hepatic cells takes place where alcohol is oxidized into acetaldehyde through ADH in cytoplasmic region, CYP450 in microsomes, and catalase in peroxisomes. The reactive acetaldehyde formed, rapidly undergoes conversion to acetate and successively forms MAA adduct (protein).

The results of alcohol metabolism include ROS production, decrease in mitochondrial glutathione, peroxidation of lipids, and increase in S-adenosylmethionine eventually leading to liver inflammation (Figure 4).

The scavenger receptors on KCs take up MAA adducts, further promoting the pro-inflammatory response. The acetaldehyde breakdown product acetate is readily released into the circulation from the liver and is then metabolized into carbon dioxide through the citric acid cycle in various organs like skeletal muscle, heart, and brain. Although
Acetate does not have any direct hepatotoxic effects, it is thought that acetate regulates the inflammatory response in individuals with AH through the up regulation of pro-inflammatory cytokines in macrophages (Shen, 2019).

Hepatic cell apoptosis

Hepatic cell apoptosis is a vital pathologic feature of human ALD. Mechanisms for apoptosis are oxidative stress, suppression of survival genes (c-Met), and stimulation of pro-apoptotic signaling molecules (TNF-α and Fas ligand).

Neutrophils Permeation

Parenchymal neutrophils permeation into the liver is an important aspect of AH. The up-regulation of several chemokines and cytokines like IL-8, CXCL1, and IL-17 in liver proportionally contributes to the permeation and severity of AH.

Alcoholic Fibrosis

Alcoholic fibrosis is characterized by the enormous accumulation of collagen and other extracellular matrix proteins. HSCs have a major important role in the progression of ALD especially fibrosis. These cells reside in the Disse as quiescent, lipid storing cells. HSCs undergo a complex activation process and thereby increase the production of extracellular matrix protein, including bone marrow-derived myofibroblasts and hepatic fibroblasts which are the characterizing features of fibrosis. (Gao and Bataller, 2011; Osna et al., 2017). HSCs activate various mechanisms like neutrophils, damaged hepatic cells, and activate KCs via numerous pro fibrogenic mediators as well as TGF-b, IL-8, TNF-a, platelet-derived growth factor and ROS. The main reason for collagen accumulation is the ROS that suppresses the action of metalloproteinases and activates metalloproteinases 1, a tissue inhibitor. They can also activate other HSC pro-fibro genic signaling pathways like ERK1, ERK2, phosphoinositide 3 kinase-Akt, and c-Jun N-terminal kinase (JNK), that subsequently induce HSC activation and fibrogenesis.

Activation of TLR4 by LPS

In individuals with ALD, the elevated serum levels of LPS are commonly observed. LPS acts by dual types of mechanisms initially stimulate KCs to produce ROS and cytokines that will result in the activation of HSCs; secondly, it will directly stimulate the HSCs through TLR4 signaling of hepatic sinusoidal endothelial cells which activate the production of proinflammatory cytokines. KCs regulate the function of HSCs.

Alcoholic Cirrhosis

Alcoholic fibrosis is transient and reversible if alcohol consumption ceases. Histopathology of the cirrhosis is characterized by regenerative nodules of liver parenchyma covered by fibrous septa. Although if the consumption of alcohol continues the chronic inflammation and sustained fibrogenesis progress, leading to the replacement of liver parenchymal cells by scar tissue that severely leads to alteration in the liver vasculature. This leads to the development of portal hypertension and/or failure of the liver (Osna et al., 2017).

Hepatocellular Carcinoma (HCC)

There are several complicated mechanisms for the progression of HCC in individuals with chronic alcohol consumption and comprise of telomere shortening, impairment of hepatocytes proliferation, alterations of the micro-environment and macro-environment that promote carcinoma survival and proliferation, loss of cell cycle checkpoints, and activation of oncogenic pathways.

Mechanism of Liver Injury From Alcohol Consumption

Binge alcohol drinking leads to severe liver diseases, which is demonstrated by increased blood levels of ALT, AST, and/or lactate dehydrogenase (LDH) and accumulation of lipid in the hepatocytes causing steatosis. There are three mechanisms involved in causing of ALD, those are: (a) acetaldehyde toxicity; (b) oxidative stress or generation of ROS; and lastly (c) provocation of the immune response of hepatocytes causing oxidative stress. This shows that oxidative stress is a major cause for ALD, therefore, defense activities against this stress are most vital in the prevention of ALD (Han et al., 2016).

Oxidative stress in ALD

Oxidative stress is the “disruption of redox signaling and control”. Hepatocytes have a variety of potential sources of ROS, which are induced or changed by chronic alcohol intake, thus leads to an augment in the production of oxidants. Hepatocytes are the principal cell type that metabolizes alcohol. It is well demonstrated that at least three discrete enzymatic pathways are implicated in the process of alcohol oxidation.

The primary pathway for alcohol metabolism is the dehydrogenase system. The alcohol is oxidized to acetaldehyde in the presence of alcohol dehydrogenase enzyme resided in the hepatocytes of the liver. The produced acetaldehyde then enter the power house of the cell i.e., mitochondria of hepatocytes wherein it gets oxidized by aldehyde dehydrogenases (ALDH) to acetate. Secondarily, the major pathway for the oxidation of alcohol is alcohol oxidizing system (MEOS), which involves NADPH-
requiring enzyme and the CYP2E1 enzyme. Lastly, and in the rare case, the alcohol is oxidized by catalase enzyme present in peroxisomes. This pathway has a minor role in alcohol metabolism because it requires the participation of hydrogen peroxide (H2O2). During the oxidation of alcohol by primary and secondary pathway as explained above, NADH or NADP+ will be produced in volume, leading to increase ROS, which is the main source of oxidative stress finally triggers ALD. Application of antioxidants especially flavonoids signifies a rational curative strategy to prevent and cure liver diseases involving oxidative stress.

Flavonoids and its Chemistry
These are the group of naturally occurring substances mainly found in plants having variable phenolic structures.

These flavonoids are chemically having 15-C skeleton composing of 2 benzene rings i.e., A and B as depicted in figure linked via a hetero cyclicpyrane ring (C) (Figure 5). They are divided into several categories like flavones (e.g., apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, and myricetin), flavanones (e.g., hesperetin, and naringenin), and others (refer Figure 6 for general structure and examples). The basic flavonoid structure is aglycone.

Antioxidant Activity and Hepatoprotective Activity of flavonoids
Flavonoids possess a number of properties; among them is the anti-oxidant activity is of prominence due to the arrangements of various functional groups as well as its nuclear structure. Its mechanisms are influenced by its configurations and functions in radical scavenging and metal ion chelation.

Mechanisms of antioxidant activity include,
1. Inhibition of ROS formation
2. Scavenging ROS
3. Protection of antioxidant defences.

Flavonoids inhibit ROS formation by regulating glutathione S-transferase, mitochondrial succinoxidase etc. Since lipid peroxidation is a result of oxidative stress, lipids are protected from oxidative damage using flavonoids.

Several flavonoids such as curcumin, apigenin, quercetin, naringenin, hesperidin, and many others have reported for their hepatoprotective activities (refer Table 1 for more details regarding mechanism of action model used and outcomes).

Metabolism of Flavonoids in Humans
The absorption of flavonoids is largely dependent on the structure of flavonoid, its properties, molecular configuration as well as solubility. Since aglycones are absorbed in a greater extent in the small intestine, flavonoids require to be converted into aglycones for better absorption.

The mechanism of transportation of hydrophilic flavonoid such as quercetin is mainly through intestinal Na+-dependent glucose cotransporter (SGLT1).

Other mechanisms suggest that flavonoid glycosides undergo hydrolysis by the enzyme lactase phloridzin hydrolase (LPH) which is present on the outer region of brush border membrane with the subsequent absorption of liberated aglycones. Its substrate specificity is dependent on the category of glycosides of the flavonoids.

In cases where the glycosides are not the substrate for the specific enzyme, they are hydrolysed by bacteria which also degrade any liberated flavonoid aglycones.

The absorption is limited in the colon, and after absorption the flavonoids are subsequently metabolized into smaller phenolic compounds by the process of glucuronidation, sulfation, or methylation and as a result, no free aglycones are identified in plasma or urine except in the case of catechins.

Flavonoids which are not absorbed in the small intestine are degraded by intestinal microflora and simultaneously undergoes break down of flavonoid ring structure. Similarly, oligomeric flavonoids undergo hydrolysis to form monomers and dimers in acidic conditions of the stomach. Larger molecules have a greater chance of reaching the colon where they are degraded and among the various classes of flavonoids, isoflavones have shown greatest bioavailability. The sugar moiety present in flavonoids is one of the major determinants of the bioavailability.

Studies have shown that consumption of green tea had resulted in an increased absorption of flavonoid content indicated by the elevation of plasma and urine levels resulting in improved plasma antioxidant levels.

Toxicity of flavonoids
Flavonoids constitute a large proportion in plants and are required to be consumed in sufficient quantities. Animal studies have shown very slight toxicity levels in pre clinical studies and the LD50 is about 2-10g per animal, which cannot be interpolated to a human dose due to impracticality and hence a minimum dose of 1mg per adult is recommended on a
daily basis. Studies carried out by Dunnick and Hailey have shown that increased intake of quercetin may result in tumor growth in mice models which was not observed in long term treatment. Moreover, as described earlier, quercetin has been reported to be anti-mutagenic In Vivo.

**CONCLUSIONS**

ALD remains a major cause of liver-related mortality in the US and worldwide, especially it is spreading rapidly in the developing countries among the indigenous people. Major advances have been made in understanding the pathogenesis of the disease at the experimental animal model level, but for researchers it has been a big challenge in finding the therapeutic targets. Moreover, Anti-Oxidative therapy mainly natural and synthetic antioxidants have shown tremendous activity against ALD by several different mechanisms including inhibition of ROS formation, scavenging ROS and protection of antioxidant defences. Numerous preclinical (in vivo and in vitro) studies have demonstrated the hepatoprotective actions of flavonoids. Flavonoids inhibit ROS formation by regulating glutathione S-transferase, mitochondrial succinoxidase etc. Since lipid peroxidation is a result of oxidative stress, lipids are protected from oxidative damage using flavonoids. Several flavonoids such as curcumin, apigenin, quercetin, naringenin, hesperidin, and many others have reported for their hepato protective activities. In this review various flavonoids with their mechanism of action, models used and outcome have been figured out in the tabular column.

Toxicity study carried out by Dunnick and Hailey has shown that increased intake of quercetin may result in tumor growth in mice models which was not observed in long term treatment. This creates a wide scope for formulating flavonoids in proper doses for the treatment of alcoholic liver disease.

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