CHAPTER II

LITERATURE REVIEWS

Alcoholic liver disease

The excessive drinking of alcohol and long-term alcohol abuse lead to alcoholic liver disease (ALD) [25]. However, the exact dose or specific dose-response relationship for ALD remains controversial [6]. There is an increase risk with the ingestion over 60-80 g/day of alcohol in a man and over 20 g/day in a woman [3] Alcohol is an underappreciated risk factor for a wide range of conditions its causes roughly 4% of all death worldwide and 5% of the global burden of disease Alcohol is also an important case of health [3]. ALD is a developing of liver damages that includes complex morphological features consisting of fatty liver (steatosis), fibrosis (hepatitis) and finally progression to cirrhosis [7]. Symptoms vary based on the severity of the disease. General symptoms can present with jaundice, liver failure, fever, and ascites, usually after years of alcohol uses [26].

Alcohol is mainly metabolized in the liver by three pathways, each located in a hepatocyte: 1. alcohol dehydrogenase (ADH) pathway in the cytosal 2. the hepatic microsomal ethanol oxidizing system (MEOS) located in the endoplasmic reticulum, with cytochrome P450 2E1-dependent pathway and 3. catalase located in the peroxisomes. The implication of each ethanol metabolism pathways produces specific metabolic and toxic disturbances which provoke the liver injury. The developments of ALD are interactions among acetaldehyde, reactive oxygen and nitrogen species, inflammatory mediators and genetic factors [27,28,29].

Alcohol metabolism

A liver is the primarily organ that alcohol is detoxified and eliminated via the three major steps consisting of (1) alcohol dehydrogenase pathway (ADH) of cytosal (2) MEOS involving an inducible ethanol cytochome P450 (2E1) located in endoplasmic reticulum and, (3) catalase located in peroxisome [27, 30]. Alcohol is oxidative metabolized to acetaldehyde by the key enzymes, ADH, cytochrome P450 2E1, and catalase to generate acetaldehyde and then acetaldehyde is metabolized by

aldehyde dehydrogenate (ALDH) to acetate. The ADH in cytoplasm is the major enzyme that has high affinity to break down alcohol to acetaldehyde. ADH-mediated oxidation of alcohol produces an excess nicotinamide adenine dinucleotide reduced form (NADH). A hydrogen is transferred from the substrate ethanol to the cofactor nicotinamide adenine dinucleotide (NAD) generating its reduced from (NADH), and acetaldehyde. Moreover, a large amount of NADH may promote fatty acid synthesis which can result in fatty liver [30]. Second, in chronic alcohol consumption, the enzyme cytochrome P450 2E1 are enhanced. They are located within liver mitochondria which are increased 4-10 fold in the liver of human and rodent after chronic ethanol exposure [31]. They are utilized in alcohol detoxification and also produce acetaldehydes [6 , 30,32]. Metabolism of ethanol by cytochrome P450 2E1 as well as ADH can generate oxidative stress in a hepatocyte. Third, ethanol metabolism is mediated by catalase. Catalase is an enzyme which is located in the peroxisomes of mammalian cells. It is capable of oxidizing ethanol *in vitro* in the presence of a hydrogen peroxide (H_2O_2) generating system [30]. These effects of catalase could be greater if a high level of H_2O_2 are produced from β-oxidation of the fatty acids in the peroxisomes [33]. However, under physiological conditions catalases appear to have a minor role.

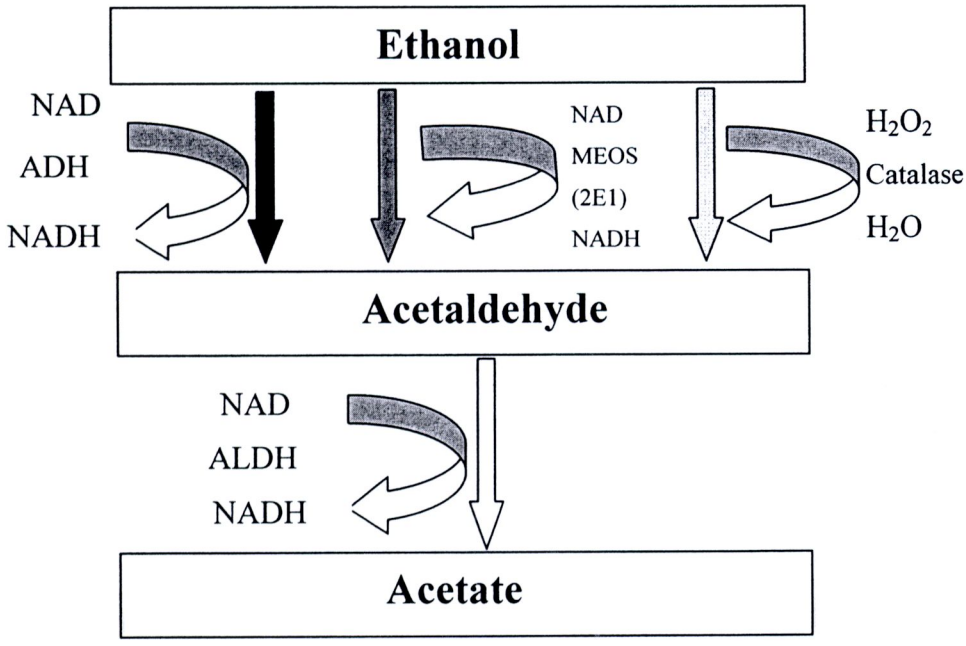


Figure 1 Ethanol metabolism in hepatocytes [34]

Pathogenesis of alcoholic liver disease

There are three main mechanisms in pathogenesis of alcoholic liver injury which compose of immunologic process, oxidative stress, and the direct toxic metabolite of alcohol generating stable protein adduct.

1. **Immunological process**: The mechanism appears to implicate the effect of alcohol on the Kupffer cells in the liver. Alcohol consumption can impair the immune response. The effect of alcohol and alcohol metabolites increase the intestinal permeability to bacterial endotoxins (lipopolysacaride, LPS) [35, 36] . Endotoxin is one of the components of the outer wall of gram negative bacteria. It has been involved in sepsis, organ failure, and lethal shock [37]. In normal condition, the gut mucosal layer is an imperfect barrier which allows small amounts of antigens and other macromolecule to pass through the intestinal wall into the blood circulation [38]. Bjarnason, I.,*et al.*, studied the possible route of entry for endotoxin, and they found that ethanol increases a membranes fluidity due to an alteration in the lipid and lipoprotein composition of the cell membrane [39]. LPS can activate Kupffer cells by binding with CD14 and then cytokines are produced. Various mediators including tumor necrosis factor alpha (TNF-α), prostaglandins, interleukins and oxygen radicals are generated [40]. These mediators have been enhanced by nuclear factor kappa B (NF-κB) activation in activated Kupffer cells and caused inflammatory responses in hepatocytes [7].

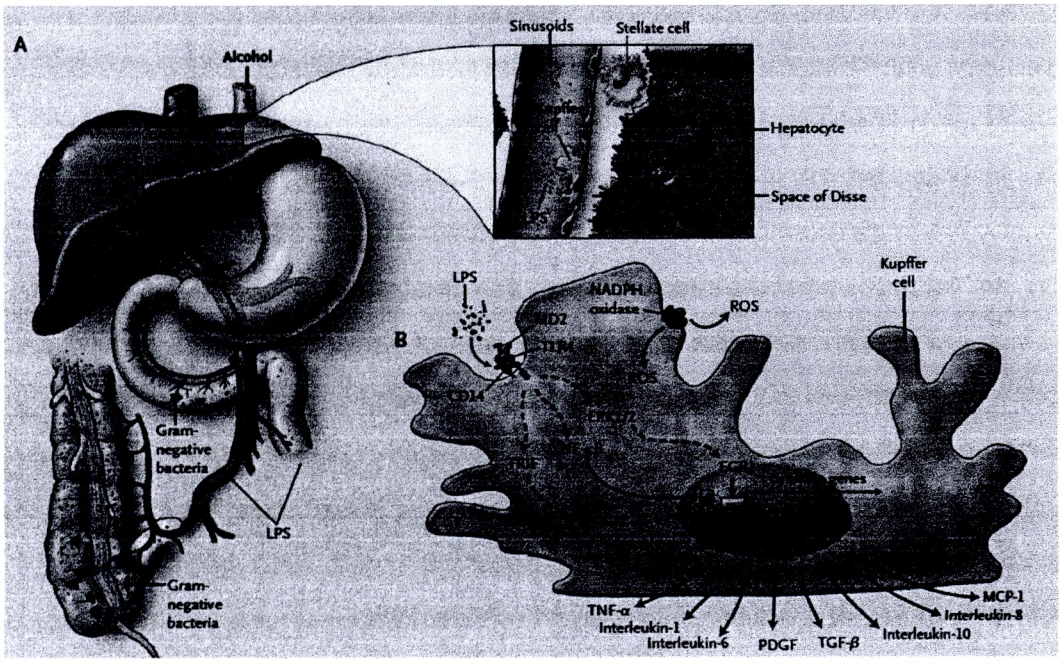


Figure 2 Mechanism of Kupffer cell activation via ethanol [37]

Note: Ethanol alters gut bacteria permeability and cause gram negative bacteria pass into blood circulation. Endotoxin from the bacteria in blood activates a Kupffer cell to produce receptors (CD-14) and pro-inflammatory cytokines

2. Oxidative stress: The second pathway is a production of oxidative stress which plays an important role in development of alcoholic liver disease [41]. Alcohol metabolism contributes to reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation [6]. Several enzymatic systems, including the cytosolic enzymes xanthine oxidase , the aldehyde oxidases and especially MEOS via cytochrome P450 2E1 (CYP2E1) have been related as sources of superoxide anion radical (O_2^-) [28]. Ethanol, similar to certain xenobiotics which are metabolized by cytochrome P450, can also be metabolized by these enzymes. Level of CYP2E1 is elevated under acute and chronic alcohol stimulation [41]. CYP2E1 has a high rate of NADPH oxidase activity which lead to the generation of large amounts of O_2^- and H_2O_2. In the presence of iron, these ROS produces powerful oxidants such as the hydroxyl radical (OH^-) [41].

to O_2^- and H_2O_2 is one of the key factors contributing to oxidative stress during ALD. Superoxide ($O_2^{\cdot-}$) is a radical which has been generated by many processes within the cell including alcohol metabolism. It is a key starting point of oxidative stress during alcohol exposure. The toxicity of ROS and RNS formations are depended on the production of $O_2^{\cdot-}$ [42]. These radicals are by themselves not potent oxidants, but they can convert ROS and RNS to generate more potent oxidants. (Figure 3) The superoxide can be catalyzed by enzyme superoxide dismutase (SOD) to generate H_2O_2. Moreover, H_2O_2 is converted to OH^- via several routes, including Haber-Weiss reactions and Fenton reactions. In addition, H_2O_2 can be change to non toxic molecule by glutathione peroxidase (GPx).

Besides $O_2^{\cdot-}$, H_2O_2 and hydroxyl anion (OH^-) are the highly reactive molecules, they can interact with lipid molecules in the cell membrane, called lipid peroxidation. Lipid peroxidation has been shown to induce disturbance of membrane organization and functional loss and modify proteins and DNA bases. It has been implicated in the pathogenesis of ALD [41].

Ethanol and nitric oxide (NO): Nitric oxide is an inorganic gas. L-arginine is the substrate and converted by the nitric oxide synthase enzyme (NOS) to generate NO [43]. NO is an odd electron species with a half-life of only a few seconds in biological system [43]. It degrades rapidly to nitrite (NO_2^-) then nitrate (NO_3^-) in solution. In the present of O_2, NO interacts with O_2^- to from peroxynitrite ($ONOO^-$), a potent oxidizing agent and other RNS radicals. These radicals have oxidizing properties which can damage a wide array of molecules in cells, including DNA and proteins. In the liver, NO is synthesized by a Ca^{2+}-dependent NOS also known as endothelial nitric oxide synthase (eNOS), which is responsible for vasodilation [44]. An inducible NOS (iNOS) activity also develops in Kupffer cells and hepatocytes. These iNOS enzymes can be stimulated by cytokines or endotoxins. The activation of cytosolic iNOS leads to formation of a large amount of NO [45]. NO levels increased by ethanol consumption have beneficial effect because of the hyperperfusion in the liver [46]. However, the increase of NO may also lead to toxicity by peroxynitrite formation. It should be considered, since ethanol consumption related to enhancement of oxygen radicals formations. Peroxynitrite anion is generated from reaction of NO with superoxide which is blamed for liver damages [46].

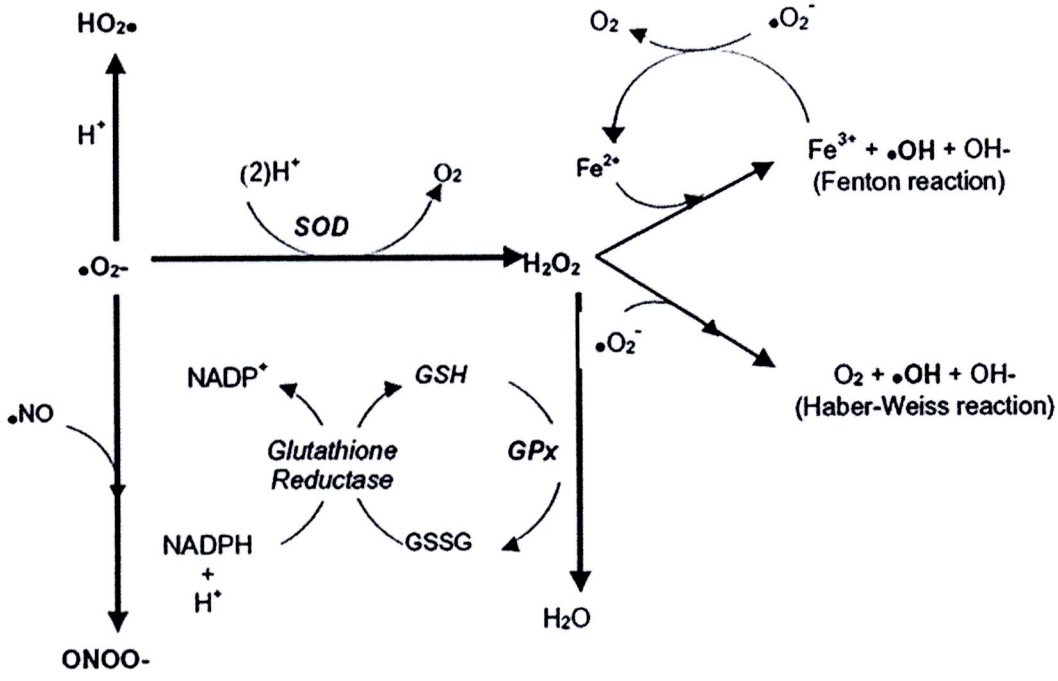


Figure 3 Schematic illustrating the formation of more toxic ROS and RNS from $O_2^{\cdot -}$, and the functions of SOD and glutathione GPx.

3. **Direct toxic metabolites generating stable protein adducts**: The third mechanism is immunogenic response from the formation of stable protein adducts. The protein adducts have been generated by ethanol metabolites, such as acetaldehyde and molondialdehyde (MDA). The interaction between liver proteins and reactive metabolites from ethanol metabolism especially the formation of protein adducts with lipid peroxidation such as MDA and 4-hydroxynonenal (HNE) can be detected in the ethanol-fed rat livers [47]. The role of a protein adduct in promoting immune reaction in ALD has emerged from the study that the antibodies specifically recognized hydroxyethyl-derived epitopes. These are detectable in chronically ethanol fed rats as well as ALD patients [42]. This mechanism induces immune reaction, resulting in necro-inflammation and/or apoptosis in tissues and possibly liver injuries [7].

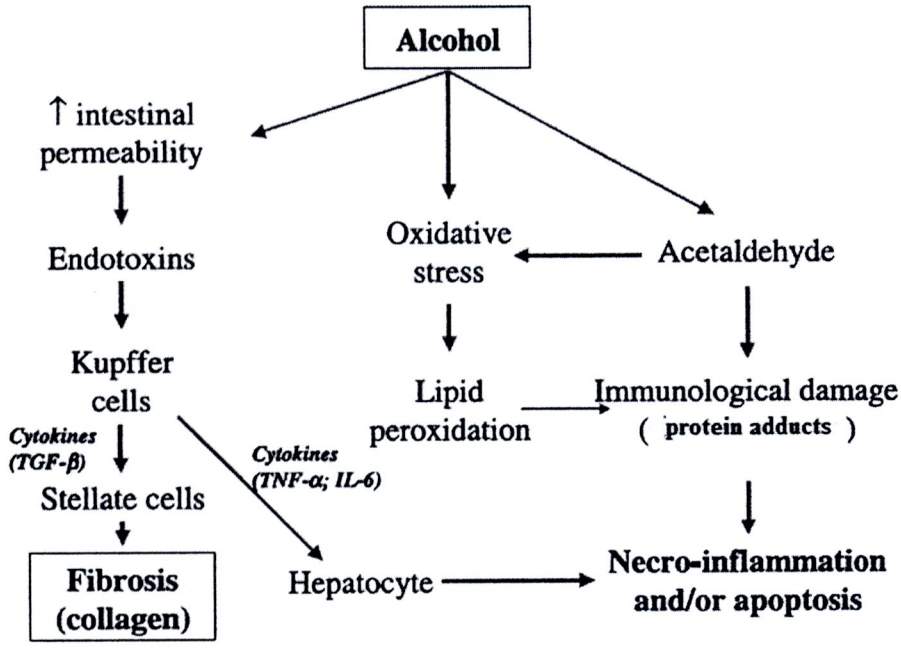


Figure 4 Diagram of the pathogenesis of alcoholic liver injury [7]

Risk factors of alcoholic liver disease: The innate background and favouring condition.

1. Gender

Liver disease progresses more rapidly in female. The evidence for this increase sensitivity in female is the sex differences in alcohol pharmacokinetics [48]. In addition, women have gastric alcohol dehydrogenase activity lower than men. The gender difference in ALD may be related to estrogen hormone. Estrogen was involved in ethanol induced liver injury by increase gut permeability and portal endotoxin, which entered to the blood and amplifying the Kupffer cells [49]. The Kupffer cells have been activated result in increased expression of endotoxin receptor CD 14 and the pro-inflamatory cytokine tumor necrosis factor alfa (TNF-α) [49, 50]. Moreover, ethanol metabolism enzymes have been regulated by estrogen hormone [49].

2. Genetic factors

Genetic predisposition to ALD has investigated. Studies of family twin and adoption studies have clearly showed that genetics play and important role in alcohol-related disorders with heritability estimated in a rang of 50-60% [51, 52]. Gene polymorphism studies found that genes encoding for enzymes metabolizing ethanol and acetaldehyde control the predisposition to alcohol dependence, sensitivity to alcohol and ALD development. These genes consist of ADH, ALDH and C2-promoter allele of the gene coding for cytochrom (CYP2E1) [53, 54]. Indeed, it is clear that polymorphisms in ALDH gene products responsible for alcohol sensitivity, especially in asian populations [55]. This evidence supports that there are the higher developments of ALD in asians with less alcohol consumption.

3. Ethnic differences

There is the different prevalence of the mortality rate for ALD between ethnic groups. In the United States, black men have higher rates of cirrhosis than whites, while hispanics present the highest cirrhosis [56]. Considerating the intake of equal amount of alcohol, hispanics and blacks are more likely to have a twofold increase in serum aspartate aminotrasferase and gamma glutamyl-traspeptidase when compared to whites [57]. However, it is not clear whether ethnic differences in the rates of ALD are due to genetic factors or the alcohol amounts intake [56, 57].

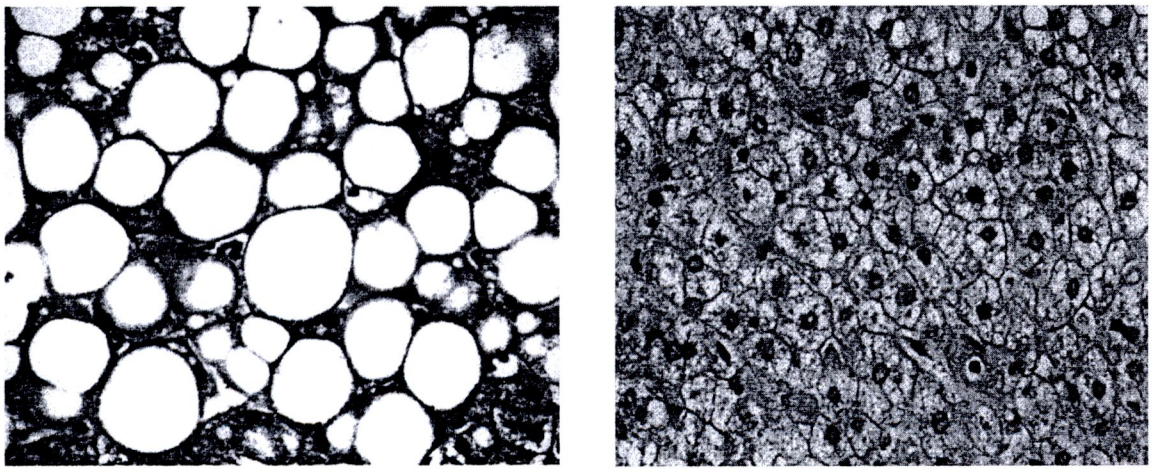
Pathologic stages of alcoholic liver disease

The histopathological features of ALD include fatty liver or steatosis, alcoholic hepatitis and cirrhosis. Steatosis and steatohepatitis represent the early phase of ALD and are the precursor stages for cirrhosis [58, 59]. In fact, a complex relationship exists between developments of ALD.

1. Fatty liver (Steatosis)

Fatty liver or steatosis usually appears in the earliest pathological process in ALD [8, 60]. There is imbalance between the storage of fat in a liver cell and its removal [61]. The morphology composed of macrovesicular steatosis characterized by the presence of only one or a fat droplet in the hepatocyte cytoplasm. In macrovesicular steatosis the fat droplets displace the hepatocyte nucleus and

cytoplasm to the edge of cell. Microvesicular steatosis is several small fat droplets per hepatocyte or mixed that accumulated triglyceride in intra-cytoplasm [9]. The small fat droplets surround rather than displace the nucleus and the nucleus maintains its central position in the hepatocyte. Macrovesicular and microvesicular fat droplets are resulted from metabolic abnormalities such as decreased fatty acid oxidation, increased triglyceride synthesis, reduced fat export and metabolism of extrahepatic fat stores [62].



A B

Figure 5 Macrovesicular and microvesicular steatosis in hepatocytes hematoxylin and eosin (H&E) stained slide.

Note: A is macrovesicular steatosis that shows one fat vacuoles in each hepatocyte. B is microvesicular steatosis which demonstrates many small fat vacuoles in each hepatocyte.

2. Alcoholic hepatitis

Alcoholic hepatitis associated with long-term heavy intake of ethanol is a syndrome of progressive inflammatory liver injury [63]. Clinical signs of alcoholic liver disease are jaundice and liver failure that commonly occurs after several years of heavy alcohol consumption (mean intake 100 g per day) [64]. Mechanisms of alcohol related alcoholic hepatitis involve in metabolism of alcohol. Under normal conditions,

the gut mucosal layer is an imperfect barrier, allowing small amounts of antigens and other macromolecules to pass through the intestinal wall into the blood [37]. Ethanol increases membrane fluidity due to alterations in the lipid and lipoprotein composition of the cell membrane. This modification of membrane fluidity due to ethanol may result in increase of transport and absorption of macromolecules [65]. Gut permeability which is the sum factors of promoting or restricting the translocation, or transfer of LPS –endotoxin from the intestinal lumen into the portal blood, appears to altered with long-term exposure to alcohol. When LPS-endotoxin enters portal blood, it binds to LPS-binding protein, a required step for inflammatory and histological responses [66].

The histological features of alcoholic hepatitis reveal hepatocellular injury characterized by balloon (swollen) which often contains amorphous eosinophilic inclusion bodies called Mallory bodies (also called alcoholic hyaline) commonly formed in alcoholic hepatitis [9, 59, 67].The formation of Mallory bodies by the result of defective hepatocellular degradative mechanisms may play a protective role in the liver [68].

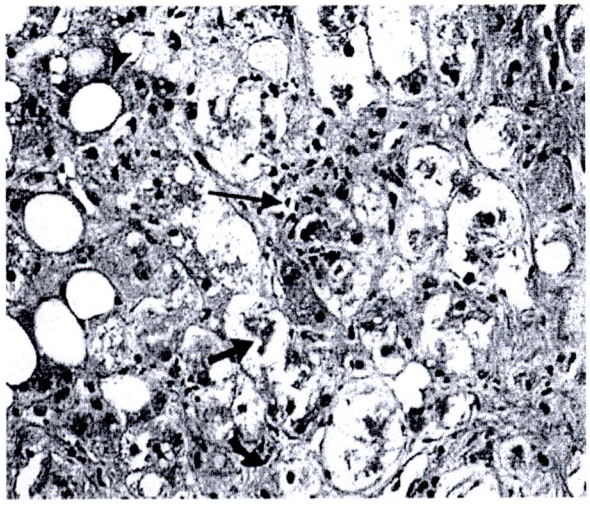


Figure 6 Alcoholic hepatitis H&E stained slide.

Note: Alcoholic hepatitis is characterized by hepatocellular injury. Some hepatocytes are displaced by fat droplets (arrow head), whereas other may contain intracellular, eosinophilic inclusion bodies called Mallary body (short arrow),

which are often surrounded by neutrophils (long arrow), and hepatocellular injury with ballooned hepatocytes (curved arrow)

3. Cirrhosis

Cirrhosis can be preceded by acute alcoholic hepatitis with fibrosis. From a morphological perspective, any kind of alcohol-induced fatty liver or alcoholic hepatitis has the potential to develop into cirrhosis [69]. The fibrous ramifications of the various portal fields make contact with one another as well as with other centrilobular fiber formation [70]. This ultimately causes pronounce fibrosis of the liver, with leading to a cirrhotic transformation when alcohol consumption continues. In general, cirrhosis occurs as an insidious process characterized by permanent liver cell necrosis and fibrillogenetic stimulation.

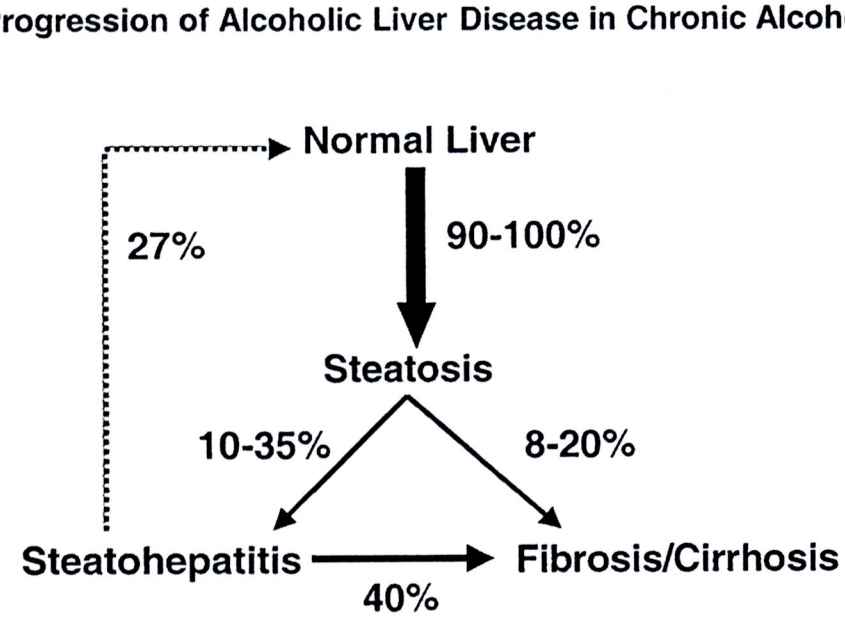


Figure 7 Progression of alcoholic liver disease in chronic alcoholics, in a sequence, ranging from steatosis (fatty liver), steatohepatitis to fibrosis /cirrhosis at the end of the spectrum [58]

Note: Steatosis and steatohepatitis are acute phases of alcoholic liver disease and it can be reversed to normal stage with discontinuing alcohol consumption

Pathological grading

The ALD comprises a morphological spectrum of liver lesions, closely resembling those seen in non-alcoholic liver disease (NALD) [71]. Hence, diagnostic criteria between ALD and NALD are similar. The numerical score is the most utilized for NALD and ALD. Normally, serum levels of liver enzymes have been used to diagnose ALD. However, AST and ALT levels can not be definitely reliable. Histological criteria have considered as a clue for the effectiveness of treatment, however the methods are complicated and needed professional pathological judgement.

Histologic scoring system for NALD (Brunt, E.M., *et al* [68], Kliner, D.E., *et al* [73])

Steatosis (0-3)

1= < 33% of hepatocytes involved

2= 33%-66% of hepatocytes involved

3= >66% of hepatocytes involved

Hepatocyte ballooning (0-2)

1= few ballooned hepatocytes (x 400 fields)

2= many ballooned hepatocytes

Lobular inflammation (0-3)

1= < 2 foci per X 200 field

2= 2-4 foci per X 200 field

3= > 4 foci per X 200 field

Fibrosis stage

1= Perisinusoidal or portal/periportal

1A : Mild perisinusoidal, zone 3 (zone 3 is located around central vein)

1B : Moderate perisinusoidal, zone 3

1C : Portal/periportal fibrosis only

2= Perisinusoidal and poral/periportal

3= Bridging fibrosis

4= Cirrhosis

Table 1 Spectrum of hepatic morphologic changes in alcoholic liver disease [59]

A. Alcoholic steatosis (fatty liver)
    1. Microvesicular steatosis
    2. Macrovesicular steatosis
    3. Mixed , microvesicular and macrovesicular steatosis
    4. Fat granulomas (lipogranulomas)
    5. Alcoholic foamy degeneration
B. Alcoholic steatohepatitis
    1. Ballooning
    2. Steatosis
    3. Inflammation
    4. Perivenular fibrosis (with or without pericellular fibrosis)
    5. Venous occlusion
C. Hemosiderosis, often mimicking hemochromatosis
D. Cirrhosis
    1. Micronodular (laennec) progressing to macronodular
E. Hepatocellular carcinoma

Symptoms of alcoholic liver disease

Clinical diagnosis of alcoholic liver disease bases on through history, physical examination and review of laboratory test. Liver function enzymes are the markers to determine ALD. The pattern of aminotransferase abnormality provides signs of alcohol induced liver injury [74]. Alcohol-induced steatosis, alcoholic hepatitis and cirrhosis may be presented individual stages development. However, the symptoms are associated with all hepatomegaly, jaundices, ascites and encephalopathy [75].

1. Ascites

In advanced liver disease symptoms, ascites is one of the most common complications and generally indicates poor prognosis. Cirrhosis causes the progressive development of portal hypertension, collateral-vein formation, and shunting of blood to systemic circulation involved in increased hepatic resistance to portal flow [76]. Ascites increase risk of spontaneous infection of ascetic fluid and difficulty breath because of the pressure of abdomen on the respiratory muscles. Moreover, ascites effect food intake with progressive malnutrition [77].

2. Jaundices

Jaundices is characterized by a yellow of the skin and sclera due to abnormally high levels of bilirubin bloodstream. High levels of bilirubin attributed from inflammation or abnormalities of hepatocytes or obstruction of bile ducts.

3. Encephalopathy

Hepatic encephalopathy is caused by accumulation in blood steam of toxic substances that are normally removed by the liver. The encephalopathy is appeared in liver failure.

Diagnostic of alcoholic liver disease

Laboratory test and questionnaires have been developed for the diagnosis of alcohol induced liver disease.

1. Questionnaire

CAGE questionnaire is an easy to remember and useful screening tool for identifying patients with alcohol dependence. Two or more positive answers indicate alcohol abuse or dependence [77].

Table 2 CAGE questionnaire

C	Have you thought you ought to **cut** down your drinking ?
A	Have people **annoyed** you by criticizing you drinking ?
G	Have you ever felt bad or **guilty** about your drinking ?
E	Have you ever had a drink first thing in the morning ? (**eye** opener)

2. Laboratory markers

Serum aminotrasferase levels have been elevated in ALD patient. The pattern of aminotrasferase abnormality level provide a hint that alcohol is likely caused of liver injury. Normally, the serum aspartate aminotrasferase (AST) levels higher than the serum alanine aminotransferase (ALT), but both will be below 300 international units per milliliter (IU/ml). The AST is two to three folds greater than the ALT level in alcoholic liver injury. Serum alkaline phosphatase level elevation can be assessed process of cholestaic pattern abnormality [37, 74]. In addition, elevated gamma glutamyl transferase (GGT) appeared to be an indicator of excessive alcohol consumption presented in ALD [75]. GGT is an enzyme in the cell membrane of many tissues including in a liver. It is also one of an indicator of excessive alcohol. Chronic alcohol consumption is often associated with hypertriglyceridaemia, hyperurecaemia, hypokalaemia and hypomagneamia, as well as elevated mean corpuscular erythrocyte volume (MCV). MCV is the index of red blood cell size.

Elevated MCV is detected in people who ingest more than 50 grams of alcohol per day [27].

3. Liver biopsy

Liver biopsy is the most sensitive and specific tool for degree of liver cell injury and hepatic fibrosis. Therefore, it remains the only way to detect dependable steatohepatitis and cirrhosis in asymptomation patients for diagnosis and treatment [78].

Principle management of alcoholic liver disease

Some studies have been reported that cirrhotic patients with alcohol hepatitis have 60% of a 4-year mortality which is worsen than many common cancers such as breast or prostate cancer. Nowadays, there is no cure for ALD. Several options have been employed to either treat the complications of ALD or attempt to treat the liver damage itself.

1. Life modifications

Abstinence from alcohol prevents further ongoing liver injury. The program of Powell, W.J and Klatskin, G showed that ALD patients with jaundices or ascites are especially beneficial from abstinence [79]. Moreover, Klatsky, A.L., *et a.* and Carrao, G., *et al.* studies showed that smoking cigarettes increase the rate of progression of fibrosis in ALD [80, 81]. Another one risk factor is obesity. Obesity is associated with development of fatty liver and non-alcoholic steatohepatitis [82]. Life style modifications in ALD are mainly issues of alcohol consumption, cigarette smoking and obesity.

2. Nutrition therapy

The patients with ALD have some degree of malnutrition [77, 83]. Importantly the degree of malnutrition correlates with the development of serious conditions such as encephalopathy, ascites and hepatorenal syndromes [75]. The mechanism by which malnutrition enhances liver damage and increases mortality in patients with ALD is unknown. There are literatures which have been suggested that alcohol drinks consume more than 30% of their calories likely to be deficient of vitamins, fats and proteins [77]. Cabre, E. *et al* demented that an enteral nutritional support improves liver functions [84]. Thus, ALD patients who voluntarily consumed

more than 3,000 kcal/day have virtually no mortality whereas those consuming less than 1,000 kcal/day have over 80% of 6-month morality [27,75].

3. Drug therapy (conventional and alternative therapies)

Nowadays, there is no the Food and Drug Administration (FDA) approved therapy for ALD. However, there are several drugs that have been widely used.

3.1 Coticosteroid

The use of corticosteroid has been studies for alcoholic hepatitis therapy. Mathurin, P., *et al* found that corticosteroids improv in severely ill alcoholic hepatitis patient at 28 days [13]. The rational for steroid use is to decrease the immune response, reduce proinflammatory cytokine production, suppress the formation of acetaldehyde adduct, and inhibit the production of collagen.

3.2 Pentoxifylline

Pentoxifylline (PTX) is a known antagonist of many proinflammatory cytokines such as TNF-α and IL-12. Normally, PTXs are used in patients with alcoholic hepatitis and alcoholic cirrhosis because of its anti-inflammatory properties [83]. PTX is a non-selective phosphodiesterase inhibitor that increases intracellular concentration of 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate and decreases production of proinflammatory chemokines/cytokines [85].

3.3 Colchicine

Colchicine has been used as a treatment for ALD. It has an anti-fibrotic effect. The mechanism of action is inhibition of collagen production, enhancement of collagenase activity and anti-inflammatory action [12].

3.4 Silymarin (Milk thistle)

Silymarin is the most widely use in the treatment of liver diseases. This agent has been extracted from ***Silybum marianum*** or milk thistle. It has anti-oxidation activity, lipid peoxidation property, anti-inflammation and anti-fibrotic effects [86]. There are many studies in hepatoprotective effect of silymarine which found to be likely beneficial in ALD. However, the indication of silymarin as a drug for liver diseases remains a controversial issue. Ferenci, P. *et al* evaluated 170 patients with cirrhosis in silymarin treatment and they observed positive effect [87]. On the

other hand, Pares, A. *et al* found that it is uneffective in study of 200 patients with alcoholic cirrhosis and hepatitis [14].

3.5 S-adenosylmethionine

Supplementation with *S*-adenosylmethionine (SAMe), a precursor for the synthesis of polyamines, choline and glutathione (GSH), has been shown to prevent the alcohol-associated with sensitivity of mitochondrial respiration, and inhibition of NO through the attenuation of iNOS induction [87]. The efficacy of SAMe in the treatment of liver cell injury has been demonstrated in several experimental models. Moto, J. M. *et al* shown that alcoholic liver cirrhosis patients who receive SAMe for 2 years had decreased liver mortality [88]. However, the approved efficacy of SAMe still need further study.

3.6 Liver transplantation

The liver transplantation is an efficacious therapy for severe liver failure associated with alcoholism. Twenty to fifty percentage of patients receive a liver transplant because of an end stage of alcoholic liver disease [89]. Quality of life appears to improve after liver transplantation due to any etiology. Therefore, the liver transplantation is a good treatment for the end-stage of ALD. However, this method has many limitations according to liver donation and expensive cost.

Turmeric (*Curcuma longa* Linn)

Class:	Liliopsida	**Subclass:**	Commelinids
Order:	Zingiberales	**Family:**	Zingiberaceae
Genus:	*Curcuma*	**Species:**	*Curcuma longa*

Figure 8 Turmeric plant [90].

Note: A height of about 3 to 5 feet, deep orange roots or tubers, and a long smooth uniform green leave with tapering at each end.

Turmeric, *Curcuma longa* Linn, the curcuma genus, belongs to the division of Mangnoliophyta, class of Liliopsida, subclass of Zingiberidae, order Zingiberales and family Zingiberaceae [17]. It is a tropical plant extensively cultivated in the tropical area of Southeast asia, India , China and tropical countries [15] including Thailand.

Turmeric is a perennial plant which grows to a height of about 3 to 5 feet and has deep orange roots or tubers. The leaves are long, smooth uniform green and

tapering at each end. Rhizomes or root tubers are powdered to obtain turmeric powders.

1. Compositions of turmeric

The rhizome of *Curcuma longa* (turmeric) has been widely used as a spice and coloring agent in many foods. *Curcuma* spp. contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), moisture (13.1%) and essential oil (5.8%) which are obtained by steam distillation of rhizomes [91]. Curcuminoids are polyphenol from the colored extract of dried powder from turmeric rhizomes [15, 16, 17, 20, 92]. It presents in 3-5% of turmeric compound. Curcuminoids contains curcumin (70%), demethoxycurcumin (18%), bisdemethoxycurcumin (5%) and recently a new substance, cyclocurcumin, found within curcuminoids [15]. The active constituents of turmeric are the flavonoid curcumin (diferuloylmetane) and various volatile oils. The best-researched active constituent is curcumin.

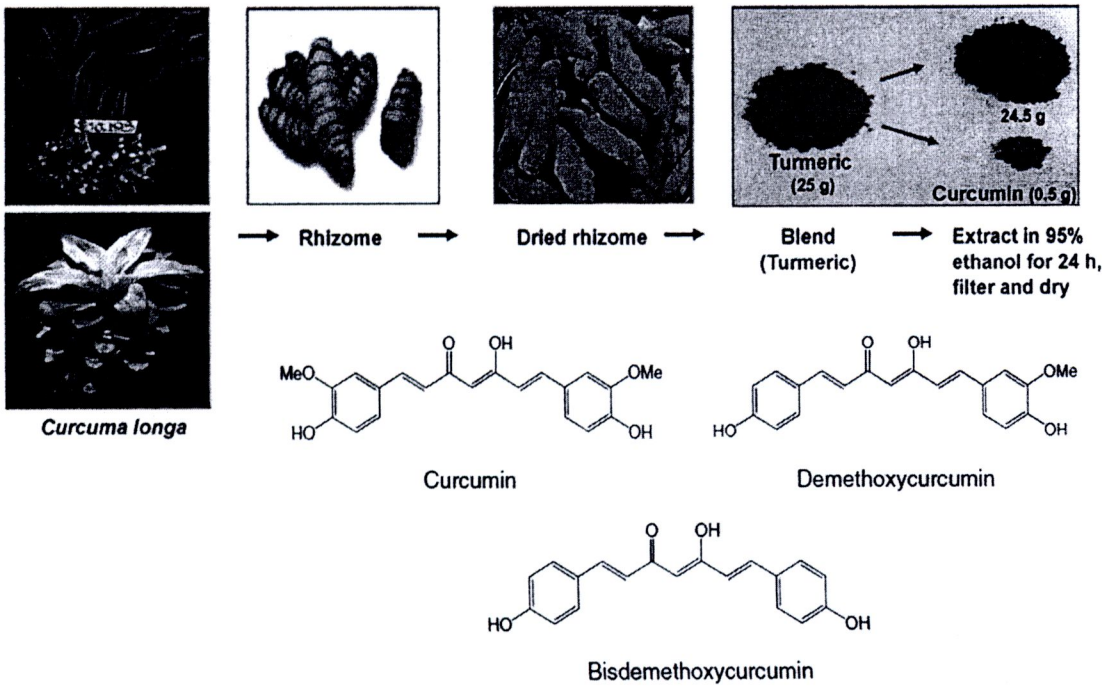


Figure 9 Turmeric plant , rhizome, turmeric compound and structures of curcumin, demethoxycurcumin and bisdemethoxycurcumin [15]

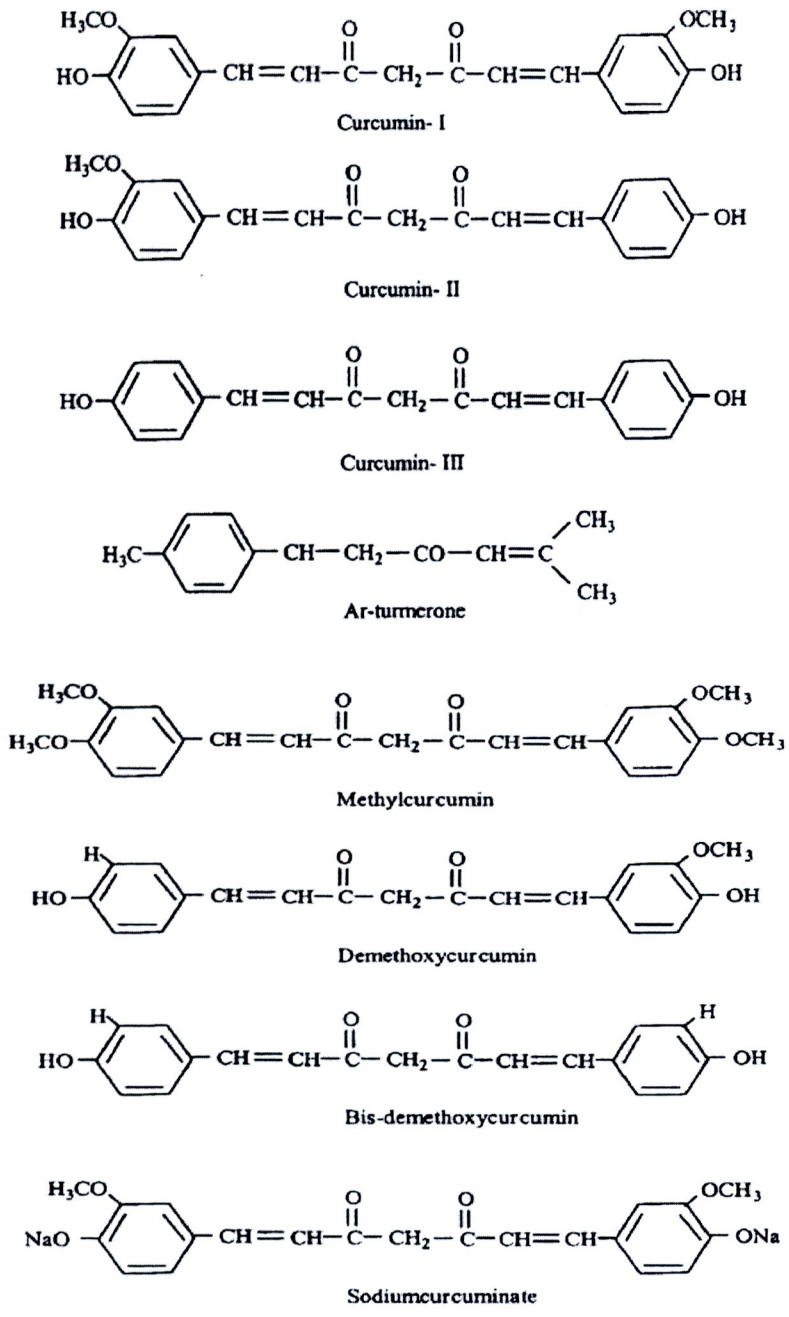


Figure 10 Structures of compositions of natural curcuminoids

2. Physical properties of curcumin

Curcumin is not soluble in water, but dissolves in ethanol, dimethylsulfoxide and acetone. $C_{21}H_{20}O_6$ is a formula of curcumin with molecular weight of 368.37 g/mol. Curcumin has a melting point of 183 °C. It is absorbed maximally at 415 to 420 nm in acetone and a 1% solution of curcumin has 1650 absorbance units [15].The stability of curcumin in presence at basic pH is unstable and degrades within 30 minutes but stable at acidic pH. Moreover, the curcumin degradation is slower in cell cultured medium containing 10% fetal calf serum and human blood, less than 20% of curcumin being degraded within 1 hour and about 50% degradation by 8 hours [16].

Pharmacokinetic studies of curcumin

The pharmacokinetics of curcumin has been widely investigated. The absorption, metabolism and tissue distribution have been studied in rodent and human. In the early study, Wahlstrom, B., *et al* investigated curcumin by oral administration in rats. They found that this compound in a dose of 1 to 5 g/kg was excreted about 75% in the feces, while negligible amount of curcumin appeared in the urine [93]. Holder, G.M., *et al* and Ravidindranath, V., *et al* studied the intravenous and intraperitoneal administration of curcumin in rodents. They found extensive amount of curcumin and metabolites in bile [91, 94]. The major biliary metabolites were glucuronidation of tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC). Curcumin is poorly absorbed from the gut. Most of the flavonoid absorption is metabolized in the intestinal mucosa and liver [93, 94]. However, there are some studies show that curcumin has beneficial effect even at the low oral bioavailability in human and rodent [95, 96, 97, 98]. Lal, B., *et al* evaluated efficacy of curcumin in chronic anterior uveitis at a dose of 375 mg three times a day for 12 weeks. They found that all the patients received curcumin alone has improved, as well as the group received antitubercular therapy plus curcumin with a response rate of 86% [96]. In 2000, Lal, B., *et al* studied efficacy of curcumin in patients who were suffering from idiopathic inflammatory orbital pseudotumours. Curcumin was administered orally at a dose of 375 mg three times a day for 6-22 months in eight patients. Five patients completed the study, out of which four recovered completely. This study suggested that curcumin

could be used as a safe and effective drug in the treatment of idiopathic inflammatory orbital pseudotumours [97]. Vareed, S.K., *et al* examined the pharmacokinetic of a curcumin preparation in healthy human volunteers 0.25 to 72 hours after a single oral 10 g in six volunteers and 12 g in another six volunteers. The results were performed by a high-performance liquid chromatography assay (HPLC). Based on the pharmacokinetic model, the area under the curve for 10 g and 12 g doses were estimated to be 35.33± 3.78 and 26±2.97 μg/mL/ h, respectively, whereas maximum concentration of curcumin (C_{max}) was 2.30 ± 0.26 and 1.73 ± 0.19 μg/ml. The time at C_{max} (t_{max}) and half life ($t_{½}$) were as estimated to be 3.29± 0.43 and 6.77 ± 0.83 hours [94]. However, the bioavailability of curcuminoids is demonstrated only with the high dose administration and pharmacological marker in clinical study is not conclusive.

Pharmacological properties of curcuminoids/curcumin

In tropical regions of Asia, turmeric has also been used as a traditional remedy for the treatment of inflammation and other diseases. Curcuminoids are one of the plant extract compound that have clinical activities. Current tradition India medicine claims the use of this powder against biliary disorders, anorexia, cough, diabetic wounds, rheumatism, sinusitis and hepatic disorders [100]. Moreover, the traditional medicines in China use *C. longa* L. in diseases which are associated with abdominal pain [92]. In Thailand, turmeric powder capsules are in the herbal medicine list of the national essential list of medicines used for abdominal disorders, and also have been promoted to use as alternate therapy in the community.

1. Anti-oxidant effects

Oxidative stress plays a major role in the pathogenesis of various diseases including alcoholic liver disease [6]. Kim, D.S., *et al* showed that the curcuminoids are more potent antioxidant than alpha-tocopherol using a 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical trapping assay in which the ability of the compound reduced a radical cation was assessed [101]. It was shown to be a potent scavenger of variety of reactive oxygen species such as superoxide anion radicals, hydroxyl radicals [102] and nitrogen dioxide radicals [103]. *Ex vivo* studies have suggested that the inducibility of magcrophage NOS activity is inhibited by curcumin [104].

The effect of curcumin on lipid peroxidation has also been studies in several models. Curcumin is good in inhibiting lipid peroxidation in rat liver microsome, erythrocytes membrane and brain homogenates [102].

2. Anti-inflammatory effects

There are articles in the literatures relating the activity of the compound extracted from *C. longa* L. being a potent inhibitor of inflammation. These substances can be classified as curcuminoids and analogues of diarylheptanoid. Both valatile oil and curcumin exhibit powerful anti-inflammatory effects [105, 106, 107]. One mechanism of anti-inflammatory activity of curcumin may be by its ability to block the production of pro-inflammatory arachidonic acid [10]. Many literatures have demonstrated that curcumin inhibits lipoxygenase (LOX) and cyclooxygenase 2 (COX-2), the enzymes that are responsible for synthesis of the pro-inflammatory leukotrienes, prostaglandins, and thromboxanes [108]. It also suppresses iNOS in activated macrophages [109], the processes that promote inflammation. Lipid peroxidation also promotes inflammation and curcumin have shown to inhibit it. The antioxidant effects of curcumin also serve to decrease the inflammations.

3. Anticarcinogenic effects

Numerous animals, *in vitro* and *in vivo* studies have demonstrated the anti-carcinogenic effect of turmeric and its flavonoid component against colon [110], breast [111], and prostate [112] cancers as well as melanoma [113]. The ability of curcumin has been showed to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis , and tumor growth [114]. The anti-carcinogenic effect of turmeric and curcumin are due to direct antioxidant and free radical scavenging, as well as their abilities to directly increase glutathione levels [15, 16]. Thereby it aids in hepatic detoxification of mutagens and carcinogens, and inhibits nitrosamine formation. The molecular basis of anti-carcinogenic and chemopreventive effects of curcumin are attributed to their effects on several targets including transcription factors, growth regulators and cellular signaling molecules [20]. Moreover, curcumin induce apoptosis in cancer cells by a variety of mechanisms as well as inhibit DNA topoisomerase II at micromolar concentrations [115]. These hint potential properties for chemotherapeutic activity in the treatment of cancer.

4. Cardiovascular effects

Curcumin decreases variety of pathological changes and thus protects damages caused by myocardial infraction [116]. Curcumin has significant hypocholesteremic effect in hypercholesteremic rats [117]. Huang, H.C., *et al* determined the effects of curcumin on the proliferation of peripheral blood mononuclear (PBMC) and vascular smooth muscle cells which are a hallmark of atherosclerosis. They found that curcumin inhibit the response of phytohemagglutinin and mixed lymphocyte reaction in human PBMC and also suppress proliferation of rabbit vascular smooth muscle cells stimulated by fetal calf serum [118].

5. Hepatoprotecive effects

In vivo and *in vitro* studies have been found that active constituents from turmeric protect animal liver from a variety of hepatotoxic substances such as carbon tetrachloride (CCl_4), galactosamine, pentobarbitol, l-cholro-2,4-dinitrobenzene,4-hydroxynonenal [119] , acetaminophen (paracetamol) [120] and alcohol [121,122]. Deshpande, U.R., *et al.,* investigated the protective effect of turmeric extracts in diet on CCl_4-treated rats. Their results indicated that a short pre-treatment of turmeric extracts show reduction in cholesterol, bilirubin, AST, ALT and alkaline phosphatase activities. In addition, concurrent treatment of turmeric extracts plus CCl_4 reduced to a greater extent of the levels of all parameters, except ALT [123]. The induction of NF-κB mediated gene expression has been implicated in the pathogenesis of ALD. Nanji, A.A., *et al* determined whether treatment with curcumin would prevent experimental ALD and evaluated the underlying mechanism in rats. Liver samples were analyzed for histopathology, lipid peroxidation, NF-κB binding, TNF-α, IL-12, monocyte chemotactic protein-1, macrophage inflammatory protein-2, COX-2, iNOS, and nitrotyrosine. The results showed that treatment with curcumin prevent both pathological and biochemical changes induced by alcohol [121]. Curcumin blocked endotoxin-mediated activation and suppression of NF-κB, the expression of cytokines, chemokines, and iNOS in Kupffer cells [121]. Naik, R.S., *et al* used liver slice culture model to demonstrate hepatoprotective effect of curcumin from ethanol cytotoxicity. They found that curcumin at 5 μM significantly reduced lipid peroxidation and lactate dehydrogenase enzyme (LDH) [124]. Xu, J., *et al* examined the effect of curcumin on hepatic stellate cells (HSC) proliferation. During liver injury, quiescent HSC became

active and proliferative. The levels of peroxisome proliferator-activated receptors-γ (PPAR-γ) were dramatically diminished along with the active HSC during liver injury [122]. Their results indicated that curcumin *in vitro* significantly inhibits proliferation of activated HSC and induced apoptosis. They also found that curcumin dramatically induces the expression of the PPAR-γ gene and activates PPAR-γ in stimulated HSC. In recently, Samuhasaneeto, S., *et al* evaluated the mechanism of curcumin attenuating inflammation and liver pathology in the early stage of ALD in rats. The results showed that curcumin treatments improve liver pathology, decrease the elevation of hepatic MDA and inhibit NF-κB activation [125].

Molecular targets of curcuminoids/ curcumin

Many study reviews have been reported that curcumin can interact to several transcription factors, cytokines, growth factors, kinases and other enzymes. The table 2 shows numerous molecules that are modulated by curcumin.

1. Several target molecule interaction

Curcumin is an efficacy molecule that interacts with numerous targets. It binds to and inhibits the activity of growth factor receptor, albumin, enzyme, metal and protein such as P-glycoprotein [126, 127], multidrug resistance protein 1, 2 (MRP1, 2), glutathione [128], protein kinase C, ATPase [129], epithelial growth factor receptor (ErbB2) as known as HER2/neu stand for " human epidermal growth factor receptor 2 [130] and alpha-1 acid glycoprotein (AGP) [131]. Curcumin inhibits tumor invasion and angiogenesis by irreversibly binding to CD13/aminopeptidase N (APN). Lipoxygenase could be inhibited with curcumin by directly binding itself [132] or binding to phosphatidylcholine and thereby the complex was inhibited [133].

2. Inhibition of transcription factor activation

Curcumin shows a potent inhibitor of various transcription factors, for instance, NF-κB, activation protein-1 (AP-1), signal transducer and activator of transcription (STAT) proteins, PPAR-γ, and β-catenin [134]. All of them regulate the genes expression that involve in tumorigenesis, inflammation, cell survival, cell proliferation, invasion, and angiogenesis [15].

3. Inhibition of multiple gene expression

Curcumin also regulates the activity of additional molecule targets which control cell adhesion, apoptosis and invasion. In this consider, curcumin has been shown as a potent inhibitor of TNF-α induced expression of intracellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule -1 (VCAM-1), and E-selectin in human umbilical vein endothelial. Furthermore, curcumin has been shown to mediate anti cancer, chemosensitive and radiosensitive effects [135].

Table 3 Lists of molecular targets of curcumin

Transcriptional factors	Enzymes
Activating protein-1	ATPase
β-Catenin	Cyclooxygenase-2
CREB-binding protein	DNA polymerase
Early growth response gene-1	Glutathione-s-trasferase
Electrophile respone element	Inducible nitric oxide syntase
Hypoxia inducible factor-1	Lipoxygenase
Notch-1	**Growth factors**
Nuclear factor-kappa B	Connective tissue growth factor
Nuclear factor 2-related factor	Epidermal growth factor
Peroxisomeproliferator-activated receptor-gamma	Fibroblast growth factor
Signal transducers and activators of transcription-1	Hepatocyte growth factor
Signal transducers and activators of transcription-2,3,4,5	Tissue factors
Wilms tumor gene 1	**Receptors**
Inflammatory cytokines	Androgen receptor
Interleukin-1, 2, 5, 6, 8, 12, 18	Epidermal growth factor receptor
Monocyte chemoattractant protein	Endothelial protein C-receptor
Migration inhibition protein	Estrogen receptor-alpha
Macrophage inflammatory protein	Histamine receptor-2
Tumor necrosis factor alpha	Low density lipoprotein-receptor

Clinical study on curcuminoids/curcumin

There are several studies to test the safety and efficacy of curcumin in humans. For example, in 1980, Deohar, S.D., *et al* studied in 18 patients to compare the antirheumatic activity between curcumin and phenylbutazone [95]. They administrated 300 mg phenylbutazone/day or 1200 mg curcumin/day for 2 weeks, and results showed that curcumin is well tolerated, and has comparable antirheumatic activity with no side effect. In 1986, Satoskar, *et al* investigated the anti-inflammatory activity of curcumin (diferuloyl methane) in 46 male patients [136]. They were administered curcumin 400 mg or lactose (placebo) 250 mg or phenylbutazone 100 mg 3 times/day for 5 days. The results suggested that curcumin is safe, and phenylbutazone and curcumin showed anti-inflammatory responses than placebo.

In 1992, Soni, K.B., *et al* studied in 10 healthy volunteers [98]. They evaluated the effect of curcumin on cholesterol levels and lipid peroxides. The researcher reported that curcumin significantly decreased total serum cholesterol. The result suggested curcumin as a chemopreventive substance against arterial diseases.

In 2000, Lal, B., *et al* reported for the first time the clinical efficacy of curcumin in the treatment of idiopathic inflammatory orbital pseudotumors [97]. Eight patients intaked curcumin at dose 375 mg/3 times/day for 6-22 months and were followed up for 2 years at 3 monthly intervals. The results showed that 4 patients recovered completely. No side effect was found in any patient and there was no recurrence. Therefore curcumin could be used as a safe and effective drug in the treatment of idiopathic inflammatory orbital pseudotumours.

In 2005, Garcea, G., *et al* investigated the effect of curcumin in 12 patients with hepatic metastasis from colorectal cancer which received 450 to 3600 mg of curcumin daily for one week prior to surgery [137]. The data showed that curcumin was poorly available, following oral administration, with low nanomolar levels of the parent compound and its glucuronide and sulphate conjugated forms found in the peripheral or portal circulation. The results suggested that doses of curcumin required to furnish hepatic levels sufficient to exert pharmacological activity are probably not feasible in humans.

Table 4 Clinical studies of curcumin in human subjects

Patients	Dose	Comments	References
18 pts (22-48 yrs)	1,200 mg/day x 2 wks	Anirheumatic effect	Deodar, S.D., *et al* (1980)
46 male pts (15-68 yrs)	400 mg; 3 times/day x 5 days	Inguinal hernia	Satosakar, R.R.,*et al* (1986)
10 volun.	500 mg/day	Serum cholesterol& & LPO	Soni, K.B.,& Kuttan, R. (1992)
40 pts.	625 mg; 4 times/day x 8 wks.	well-torarated Antiviral study	James, J.S. (1994)
53 pts.	375 mg; 3 times/day	Chronic anterior Uveitis	Lal, B., *et al* (1999)
8 pts	375 mg; 3 times/day 6-22 months	Idiopathic inflammatory. orbital pseudotumors	Lal, B., *et al* (2000)
25 pts.	500 mg-12,000 mg/day	five high-risk cancers	Cheng, A.L., *et al* (2001)
15 pts.	36-180 mg 4 months	Colorectal cancer decrease Serum GST	Sharma, R.A., *et al* (2001)
12 pts.	450-3,600 mg/day x1 wks	Hepatic metastasis of colorectal cancer	Garcea, G., *et al* (2004)
15 pts.	450-3,600 mg/day x 4 months	Advanced colorectal cancer	Sharma, R.A., *et al* (2004)
12 pts.	3.6, 1.8, or 0.45 g/day 7 days	Colorectal cancer	Garcea, G., *et al* (2005)
3-6/cohort	-	Colon cancer	Brenner, D.E., *et al* (2005)
6 volun	150 mg x 2 wks and 210 mg single dose	Pharmacokinetic of curcumin nanoparticle	Kanai, M. *et al* (2011)

Note: (*Abbreviations*: d: day, pts: patients, volun: volunteer, wks: weeks, yrs: years, LPO: lipid peroxidation)