

ROLE OF ANTIOXIDANT IN THE MANAGEMENT OF HEPATIC COMPLICATIONS

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Summary

Liver is one of largest and vital organ of human body and is vulnerable for tissue insult continuously. Drug induced hepatotoxicity (DIH) is one of major concern which limit the therapy and drugs use. About 2 % of all causes of jaundice in hospitalized patients are drug induced. About a quarter of cases of fulminant hepatic failure are thought to be drug related. Early detection is needed to identify a drug related hepatic reaction. Severity is greatly increased if the drug is continued after symptoms or if the serum liver enzymes continue to rise. The antioxidant system of our body tries to maintain homeostasis but usually fails and leads to hepatic complication. The DIH can be effectively managed by use of natural antioxidants in form of food supplements or polyherbal preparation. Hence, present literature of review discusses the role of antioxidants in management of hepatic complications.

Keywords: Antioxidant, Hepatic complications, Silymarin

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Introduction

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions (1). Liver disease is still a world wide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complimentary and alternative medicine (CAM). Many traditional remedies employ herbal drugs for the treatment of liver ailments (2, 3, 4, 5).

Liver disease is a broad term describing number of diseases affecting the liver. Many are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakup of the hemoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile.

- Hepatitis, inflammation of the liver, caused mainly by various viruses but also by some poisons, autoimmunity or hereditary conditions.
- Cirrhosis is the formation of fibrous tissue in the liver, replacing dead liver cells. The death of the liver cells can for example be caused by viral hepatitis, alcoholism or contact with other liver-toxic chemicals.
- Haemochromatosis, a hereditary disease causing the accumulation of iron in the body, eventually leading to liver damage.
- Wilson's disease, a hereditary disease which causes the body to retain copper.
- Budd-Chiari syndrome, obstruction of the hepatic vein.
- Gilbert's syndrome, a genetic disorder of bilirubin metabolism, found in about 5% of the population. (6)
- Non-alcoholic fatty liver diseases.

Hepatitis implies injury to the liver characterized by the presence of inflammatory cells in the tissue of the organ. Hepatitis can also be due to toxins (alcohol), other infections or from autoimmune process. Among the different types of hepatitis like alcoholic, viral, ischemic hepatitis the drug induced hepatitis is the one which mainly limit the use of drugs in different therapy. A large number of categories of drugs, if not all are well known for causing hepatotoxicity some of them are enlisted in table 1. (7)

Table 1: The example of different category of drug causing hepatotoxicity.

| Category | Examples of drugs |
|-----------------------------------|---|
| Antidepressant | Amitriptyline |
| Antiarrhythmic | Amiodarone |
| Anesthetic gas | Halothane |
| NSAIDs | Ibuprofen and indomethacin |
| Tuberculosis-specific antibiotics | Isoniazid (INH), rifampicin, and pyrazinamide |
| Antifungal | Ketoconazole |
| Antihistamine | Loratadine |
| Antihypertensive | Methyldopa, Nifedipine |
| Immune suppressant | Methotrexate |
| Tetracycline antibiotic | Minocycline |
| Antibiotic | Nitrofurantoin |
| Antiepileptics | Phenytoin and valproic acid |
| Antidiabetic, | Troglitazone |
| Antiretroviral | Zidovudine |
| Anticancer | Azathioprine (8) |

Mechanisms of hepatotoxicity:

Certain drugs will produce predictable liver damage in the majority of cases after overdoses. In some cases the mechanism may involve the parent compound; in others a metabolite may be responsible. Direct cytotoxicity is known to be the underlying cause of liver damage in certain cases whereas in others immunological mechanisms or even a mixture of both cytotoxicity and immunogenicity may be involved. The various mechanisms according to the type of injury are as follows which limit the use of drug.

1. Interference with bilirubin transport and conjugation:-

A number of drugs interfere with bilirubin transport and lead to elevated plasma bilirubin or hyperbilirubinaemia. (9) Novobiocin inhibits Uridyl diphosphate (UDP) glucuronosyl transferase and may lead to elevated plasma levels of unconjugated bilirubin especially in neonates. (10)

Rifampicin: Rifampicin, the antibiotic used in the treatment of tuberculosis inhibits both uptake and excretion of bilirubin in a dose related manner, giving rise to elevated plasma levels of both conjugated and unconjugated bilirubin. This is due to blockade of uptake at the plasma membrane of the hepatocyte. (9, 11) As well as causing hyperbilirubinaemia, rifampicin may also cause overt hepatic damage. (12) The lesion is characterised as hepatocellular with centrilobular necrosis which may be accompanied by cholestasis. Effects of rifampicin include interference with DNA synthesis and the ability to induce hepatic microsomal enzymes and cause proliferation of the smooth endoplasmic reticulum.

2. Cytotoxic injury:-

Direct, overt damage to hepatic parenchyma may be caused by a number of drugs. It may have a variety of underlying mechanisms.

Paracetamol: This minor analgesic causes predictable centrilobular hepatic necrosis in both experimental animals and man after overdoses. (13, 14) Doses of 10 g may lead to liver damage, and doses greater than 15 g may be sufficient for fatal hepatic damage. Liver damage may be detected as raised serum transaminases, Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) which may reach levels of 5000 IU/L. (15) However, bilirubin levels may be only moderately elevated. The reactive metabolite *N*-acetyl-*p*-benzoquinone imine then reacts covalently with cellular macromolecules as revealed by measurement of covalent binding of radiolabelled paracetamol to hepatic protein and autoradiographic studies which indicate binding primarily in necrotic areas (16) Nucleophiles and increasing glutathione levels protects against the hepatic injury. (17)

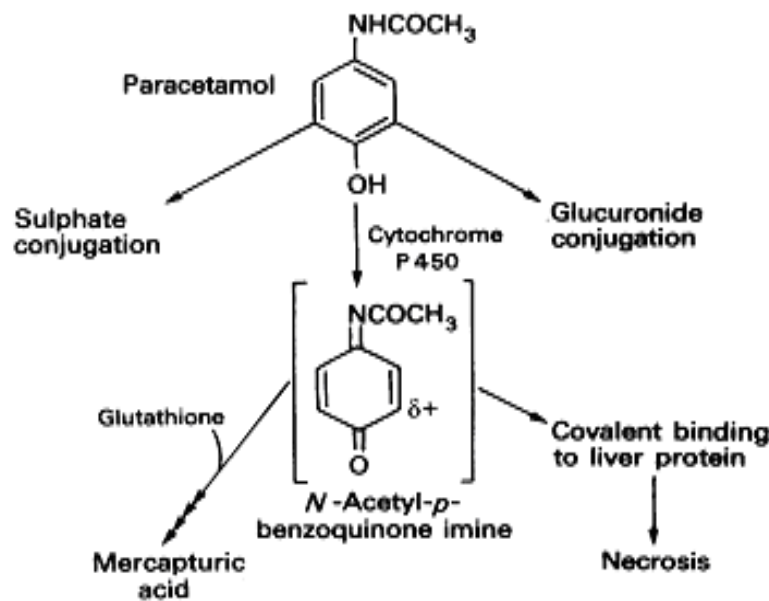


Figure 2: Metabolism of paracetamol showing proposed metabolic activation and its involvement in the toxicity. (16)

3. Cholestatic injury:

This type of hepatic toxicity may be seen with a number of drugs, and may be either the mild, canalicular form or the more severe hepatocanalicular variety.

Chlorpromazine: This major tranquilizer is an important cause of drug-induced jaundice. The incidence is estimated at between 0.5 and 1% of recipients of therapeutic doses (18) although up to 50 % of patients receiving large doses may have minor abnormalities of liver function. (19) Jaundice commonly develops after 3 weeks of therapy, with often the development of fever, itching, abdominal pain, nausea and anorexia. This is often similar to extrahepatic obstructive jaundice, with elevated serum cholesterol and alkaline phosphatase levels more than 4 times normal. SGOT and SGPT levels may also be moderately raised. Chlorpromazine is an amphipathic, cationic tertiary amine detergent. It is highly concentrated in bile during biliary excretion and the concentration in bile may exceed that intrinsically toxic in vitro to cell membranes. (20)

Anabolic steroids: Steroids with an alkyl group at C₁₇ (Figure 3) such as methyl testosterone may give rise to mild hepatotoxicity (21). This is normally a cholestatic injury generally without parenchymal damage (canalicular type). Thus alkaline phosphatase is only slightly raised (2 times normal) as are transaminases SGOT but jaundice may be marked, with elevated bilirubin levels (30 mg/100ml).

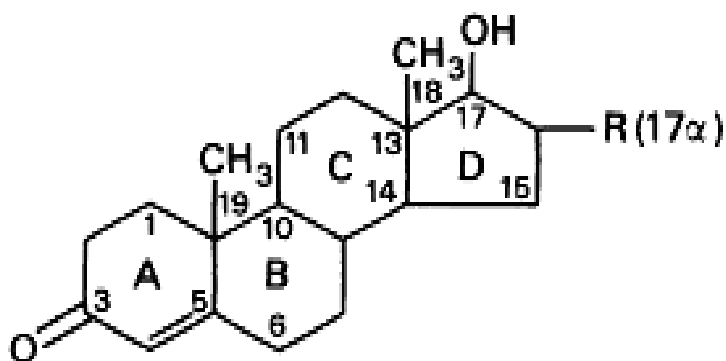


Figure 3: Basic structure of steroids related to testosterone. For testosterone R = H.

There are precise structural requirements for the steroids which produce this hepatic damage. Thus a C₁₇-methyl substituent is more active than an ethyl or vinyl group (22) keto group at C₃ confers greater activity than a hydroxyl group and saturation of the A ring of the steroid reduces its ability to cause hepatic dysfunction. (23) Although the exact mechanism of hepatotoxicity is obscure, anabolic steroids clearly cause a dose related blockage of bile secretion.

4. Mixed cytotoxic / cholestatic injury:

This type of liver injury covers damage with varying proportions of cytotoxic and cholestatic involvement. For example chlorpromazine may cause mixed hepatocanalicular jaundice with parenchymal injury as well as cholestasis. Conversely, p-aminosalicylic acid may cause mixed hepatocellular liver injury.

5. Fatty liver (steatosis):

Tetracycline: This antibiotic may cause fatty liver after large (1.5g/day) intravenous doses. (23) Histologically hepatocytes are packed with small fat droplets. This microvesicular steatosis occurs initially in the centrilobular area. There is little inflammation, necrosis or cholestasis. The major effect seems to be inhibition of transport of lipid out of the hepatocyte. This effect may be due to the inhibition of protein synthesis caused by tetracycline which will inhibit the production of the apolipoprotein complex involved in transport of the very low density lipoprotein (VLDL) out of the hepatocyte. (24) Alternative or additional mechanisms may involve decreased fatty acid oxidation, increased triglyceride uptake or increased fatty acid uptake. (Figure 4)

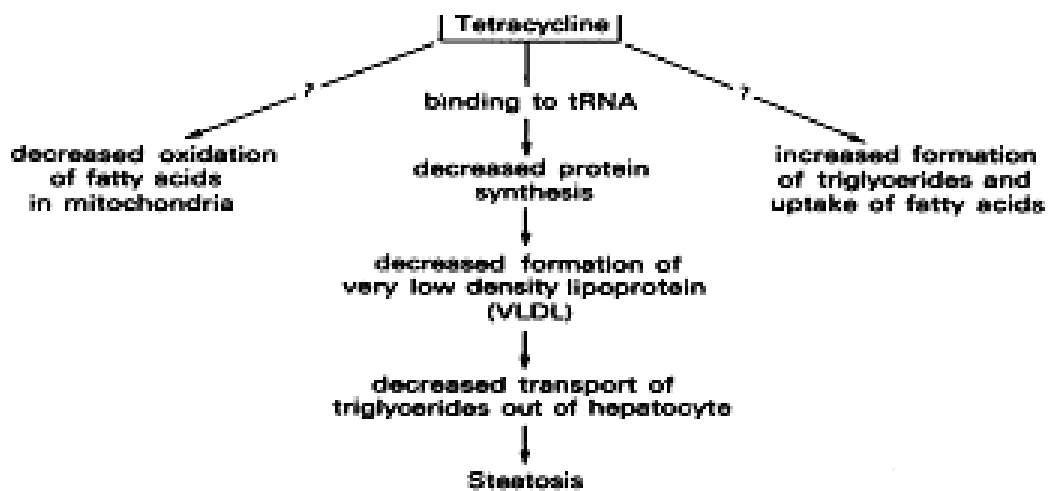


Figure 4: Postulated mechanism for tetracycline induced fatty liver

6. Chronic active hepatitis, cirrhosis and sub-acute necrosis:

Chronic active hepatitis, sometimes associated with cirrhosis is associated with the use of a number of drugs such as oxyphenisatin, α -methyl dopa, nitrofurantoin and isoniazid. Isoniazid has been chosen as the example because the mechanism is at least partially understood.

Isoniazid: Long term administration of the antitubercular drug isoniazid leads to hepatic dysfunction in a significant proportion of recipients (10-20%). However, some 0.1-1% of patients develops severe hepatic injury. (25) Although this is generally acute hepatocellular damage, in about 10% of cases chronic active hepatitis may develop with or without cirrhosis. SGOT and SGPT values are elevated, (up to 10-20 times normal), but alkaline phosphatase levels are only moderately raised, except in about 10% of patients when mixed hepatocellular jaundice occurs, giving 4 times normal values for alkaline phosphatase. Serum bilirubin may therefore also be raised. Preexisting liver dysfunction, such as alcoholic cirrhosis, also increases susceptibility. (26)

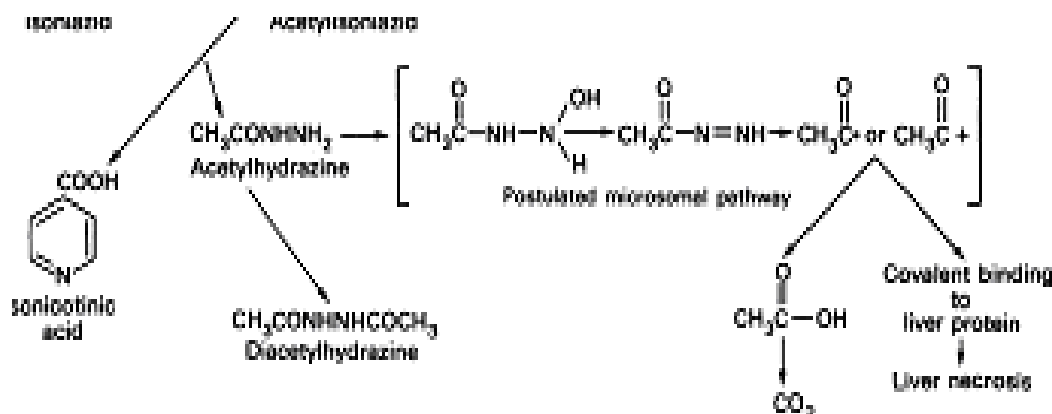


Figure 5: Metabolism of isoniazid showing proposed metabolic activation and its involvement in the toxicity.

Also isoniazid hepatotoxicity may be reproduced in experimental animals. (27) The mechanism of isoniazid induced hepatic damage involves production of a toxic metabolite. The reports of some finding suggested that rapid acetylation might be a predisposing factor as it was reasoned that this would produce more of the metabolite acetylisoniazid and hence more acetylhydrazine (Figure 5). Acetylisoniazid and especially acetylhydrazine are extremely hepatotoxic causing centrilobular hepatic necrosis, in experimental animals in which the microsomal enzymes are induced by phenobarbitone (28). The hepatotoxicity of acetylisoniazid depends on its metabolism to acetylhydrazine. This metabolite in turn is metabolically activated by the microsomal enzymes to a reactive acylating species which reacts covalently with liver protein. (29) The role of the acetylator phenotype in this is complex, as studies in human volunteers indicated that although rapid acetylators produced more potentially toxic acetylhydrazine, this was then further acetylated to non-toxic diacetylhydrazine. (30)

7. Phospholipidosis:

This syndrome may be caused by a number of different drugs, and various organs may be affected. Hepatic phospholipidosis has been caused by the drug Coralgil, a coronary dilator, in Japan. (31) The features of this form of hepatic damage are an accumulation of phospholipids in hepatocytes, bile duct proliferation and inflammation in the portal area. SGOT and SGPT values may be elevated. The lesion may progress to liver cirrhosis and have a fatal outcome. The mechanism is thought to involve the formation of complexes between lipid micelles or liposomes, and the drug in question. (32)

8. Liver tumours:

Anabolic steroids have been implicated as responsible for primary hepatocellular carcinomas and adenomas. Similarly use of contraceptive steroids has been connected with liver tumours, particularly the oestrogenic components. (33)The mechanism(s) is unknown although interference with the metabolism of foreign compounds or bile salt derivatives so as to increase their tumourgenicity has been suggested. (34)

A free-radical is simply defined as any species capable of independent existence that contains one or more unpaired electrons. It may be superoxide (O_2^- , an oxygen centred radical), thiyl ($RS\cdot$, a sulphur-centred radical), trichloromethyl ($CCl_3\cdot$, a carbon centred radical) or nitric oxide ($NO\cdot$) in which the unpaired electron is delocalized between both atoms. The O_2^- , hydroxyl radicals ($\cdot OH$) and other reactive oxygen species (ROS) such as H_2O_2 are continuously produced in vivo. (35) Continuous interaction of the animal physiological systems with these free radicals generated either indigenously or inhaled/ingested from exogenous sources therefore, lead to excess load of free radicals and cause cumulative damage of protein, lipid, DNA, carbohydrates and membrane, resulting in so-called oxidative stress. Therefore, living creatures have evolved a highly complicated defence system with antioxidants composed of enzymes and vitamins against oxidative stress in the course of their evolution. These defence systems are mainly classified as (i) suppression of generation of ROS, (ii) scavenging of ROS, (iii) clearance, repairing and reconstitution of damage and (iv) induction of antioxidant proteins and enzymes. Therefore, any additional burden of free radicals, either from an indigenous or exogenous source on the animal or human physiological system can tip free radical (prooxidant) and anti-free radical (antioxidant) s balance leading to oxidative stress. (1)The oxidative stress, defined as the imbalance between oxidants and antioxidants in favour of the former potentially leading to damage has been suggested to be the cause of aging and various human diseases. (36)

In food, antioxidants have been defined as a substance that in small quantities is able to prevent or greatly retard the oxidation of easily oxidisable materials such as fats.(37) However in biological systems the definition for antioxidants has been extended to any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate like lipids, proteins, DNA, & carbohydrates.(38,39) Over the past three decades, free-radical theory has greatly stimulated interest in the role of dietary antioxidants in preventing many human diseases, including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration and diabetes. These wide varieties of chronic inflammatory diseases form the basis for development of antioxidant based therapeutics. Regardless of their initiating pathological events, these diseases share a series of steps that lead to a common mechanistic pathway of oxidative stress through regulatory oxidative signals. (40)

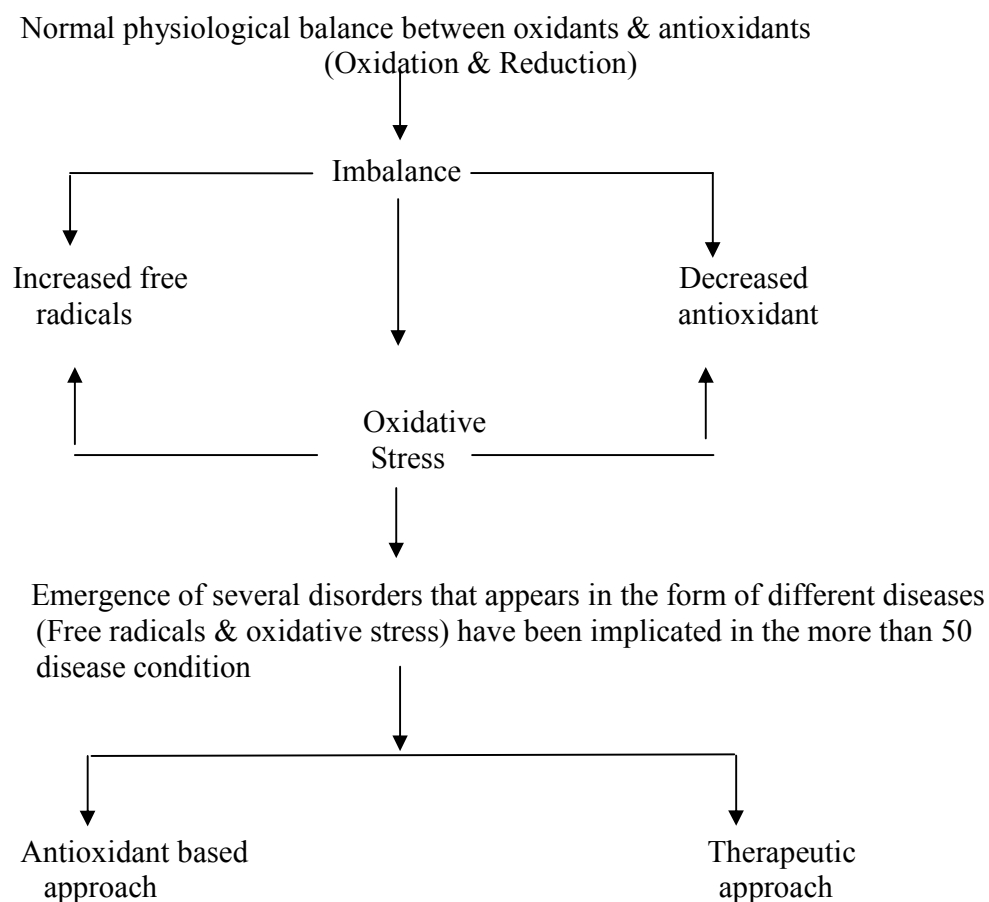


Figure 1: Oxidative stress occurs due either to the increased generation of free radicals or compromised and/or decreased antioxidant defence. This imbalance can be managed by exogenous supply of antioxidant rich nutrition, natural and/or synthetic antioxidant principles-based therapeutic preparations.

Liver is the most important organ of metabolism and excretion. About 20, 000 deaths occur every year due to liver diseases. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2, 50,000 new cases each year. Although viruses are the main cause of liver diseases, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon. Modern drug have very little to offer for alleviation of hepatic ailments whereas most important representatives of phytoconstituents used for liver disases chiefly on regional basis include drugs like silymarine(*Silybum marianum*) and catechin (*Anacardium occidentale*) in Europe glycyrrhizin(*Glycyrrhiza glabra*) in Japan and chizandrins(*Schizandra chinensis*) in china.(41)In India over 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. Scrutiny of the literature indicates that 160 phytoconstituents from 101 plants families have antihepatotoxic activity. (42) The plants which having antioxidant potential are enlisted in Table 3.

Table 3: Plants having antioxidant potential

| No | Name of plant | Family | Uses | Reference |
|-----|-------------------------------|-------------------|--|-----------|
| 1. | <i>Aegiceras corniculatum</i> | Aegicerataceae | Antiinflammatory, hepatoprotective, antioxidant | (43) |
| 2. | <i>Terminalia chebula</i> | Combretsceae | Hepatoprotective, antioxidant | (44) |
| 3. | <i>Ichnocarpus frutescens</i> | Apocynaceae | Hepatoprotective, antioxidant | (45) |
| 4. | <i>Cassia siamea</i> | Fabaceae | Insomnia ,asthma, antioxidant | (46) |
| 5. | <i>Bacopa monnieri</i> | Schrophulariaceae | Hepatoprotective, antioxidant | (47) |
| 6. | <i>Glinus lotoides</i> | Aizoceae | Anticholesterolemic, hepatoprotective, antioxidant | (48) |
| 7. | <i>Solanum fastigiatum</i> | Solanaceae | Hepatoprotective, antioxidant | (49) |
| 8. | <i>Euphorbia antiquorum</i> | Euphorbiaceae | Hepatoprotective,antioxidant | (50) |
| 9. | <i>Diospyros malabarica</i> | Ebenaceae | Hepatoprotective, antioxidant | (51) |
| 10. | <i>Azadirachta indica</i> | Meliaceae | Hepatoprotective, antioxidant | (52) |
| 11. | <i>Acalypha racemosa</i> | Euphorbiaceae | Hepatoprotective, antioxidant | (53) |
| 13. | <i>Pisonia aculeata</i> | Nyctaginaceae | Hepatoprotective, antioxidant | (54) |
| 14. | <i>Chamomile capitula</i> | Asteraceae | Hepatoprotective, antioxidant | (55) |
| 15. | <i>Epaltes divaricata</i> | Compositae | Hepatoprotective, antioxidant | (56) |
| 16. | <i>Teucrium polium</i> | Lamiaceae | Hepatoprotective, antioxidant | (57) |
| 17. | <i>Hygrophila spinosa</i> | Acathaceae | Hepatoprotective,antioxidant | (58) |
| 18. | <i>Cassia occidentalis</i> | Leguminosae | Hepatoprotective,antioxidant | (58,59) |
| 19. | <i>Allium sativum</i> | Alliaceae | Hypolipidaemic, antioxidant | (60) |
| 20. | <i>Phyllanthus niruri</i> | Euphorbiaceae | Hepatoprotective,antioxidant | (61) |
| 21. | <i>Smilax chinensis</i> | Liliaceae | Hepatoprotective,antioxidant | (62) |
| 22. | <i>Capparis spinosa</i> | Capparidaceae | Hepatoprotective,antioxidant | (63) |
| 23. | <i>Annona squamosa</i> | annonaceae | Hepatoprotective,antioxidant | (64) |
| 25. | <i>Punica granatum</i> | Punicaceae | Hepatoprotective,antioxidant | (65) |

Silymarin:-

Silymarin, a flavonolignan from ‘milk thistle’ *Silybum marianum* (Family: Asteraceae/Compositae) is the oldest and thoroughly researched plants in the treatment of liver diseases. (66) Silymarin consists of four flavonolignan isomers namely- silybin, isosilybin, silydianin and silychristin. Among them, silybin being the most active and commonly used. It acts by antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms.

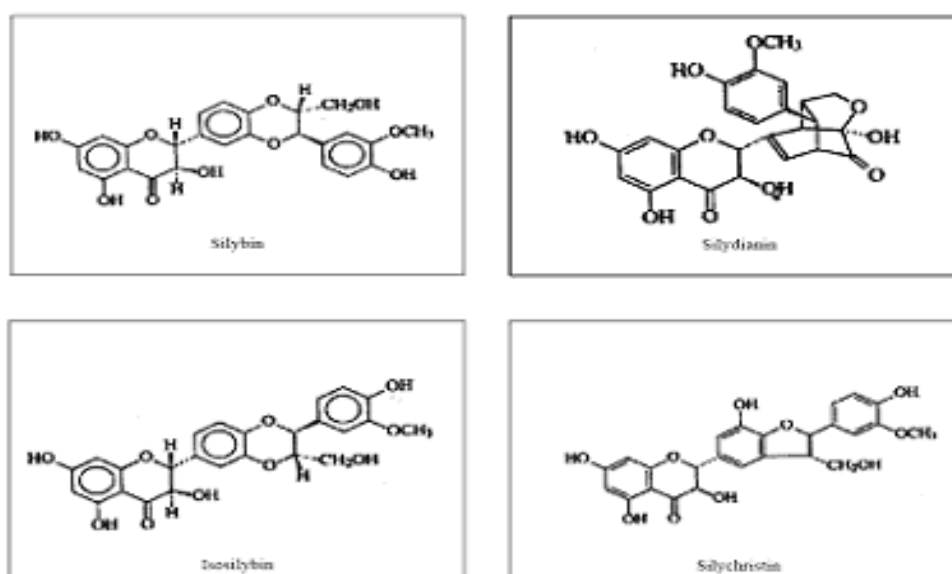


Figure 6: Structures of flavonolignan isomers of silymarin.

MECHANISM OF ACTION:

The preclinical studies using different hepatotoxic substances showed that silymarin has multiple actions as a hepatoprotective agent. The antioxidant property and cell-regenerating functions as a result of increased protein synthesis are considered as most important.

(i) Antioxidant properties: Free radicals, including the superoxide radical, hydroxyl radical ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), and lipid peroxide radicals have been implicated in liver diseases. (67) These reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to xenobiotics. (68) The mechanism of free radical damage include ROS-induced peroxidation of polyunsaturated fatty acid in the cell membrane bilayer, which causes a chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane lipids and proteins. Subsequently cell contents including DNA, RNA, and other cellular components are damaged. (69)

The cytoprotective effects of silymarin are mainly attributable to its antioxidant and free radical scavenging properties. Silymarin can also interact directly with cell membrane components to prevent any abnormalities in the content of lipid fraction responsible for maintaining normal fluidity. (70)

(ii) Stimulation of protein synthesis: Silymarin can enter inside the nucleus and act on RNA polymerase enzymes resulting in increased ribosomal formation. This in turn hastens protein and DNA synthesis. (71) This action has important therapeutic implications in the repair of damaged hepatocytes and restoration of normal functions of liver.

(iii) Anti-inflammatory actions: The inhibitory effect on 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis is a pivotal pharmacological property of silymarin. Leukotriene (B₄) synthesis was reduced while prostaglandin (E₂) synthesis was not affected at higher concentrations of use of silibinin. (72) A study which evaluated the action of silibinin in isolated Kupffer cells indicated a strong inhibitory effect on leukotriene B₄ (LTB₄) formation with the IC₅₀ value of 15 µmoles/l. But no effect was observed on tumour necrosis factor-alpha (TNF-α) formation. (73) The NF-κB is a key regulator of inflammatory and immune reactions. Silymarin is found to suppress both NF-κB DNA binding activity and its dependent gene expression induced by okadaic acid in the hepatoma cell line HEP G₂. But the NF-κB activation induced by TNF-α was not affected by silymarin, demonstrating a pathway dependent inhibition by silymarin. (74)

(iv) Antifibrotic action: Liver fibrosis can result in remodeling of liver architecture leading to hepatic insufficiency, portal hypertension and hepatic encephalopathy. These processes involve complex interplay of cells and mediators. (75) In the initial phase there will be proliferation of hepatic parenchymal cells. The conversion of hepatic stellate cells (HSC) into myofibroblast is considered as the central event in fibrogenesis. Silymarin inhibits NF-κB and also retards HSC activation. It also inhibits protein kinases and other kinases involved in signal transduction and may interact with intracellular signaling pathways. (76)

In conclusion it is clear that liver diseases should be carefully managed because of its high incidences and high rate of occurrences, drug induced liver complications are major cause, which can be properly managed by use of the antioxidants from food origin. The different antioxidant herbs are widely used traditionally a systematic and scientific evaluations of these herbs can open new avenues for management of liver complications. Hence role antioxidant in management of hepatic complications is an effective tool which needed to be redefining.

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