Introduction
LIVER

The largest organ in the body is the “LIVER” and it also serves as the primary metabolic organ of the body. Though the liver is made up of different cells like hepatocytes, endothelial, kupffer and stellate cells are the most predominant with significant functions. Another most important unique feature of the liver is its ability to regenerate. Well grown-up liver (i.e. Adult) is the principle organ in-charge of detoxifying and metabolizing, exogeneous/endogenous compounds, rendering them more hydrophilic, which frequently influence their intensity and action. 

Figure 1.1: Liver Detoxification pathways
Liver diseases are the real therapeutic issues confronted by the individuals everywhere throughout the world. The epidemiological study shows that around 20,000 deaths happen consistently because of liver disorders. In Africa and Asia, the fundamental driver of liver diseases are infections by virus and parasite, while in Europe and in North America, a noteworthy reason is alcohol abuse. Liver diseases are chiefly brought about by lethal chemicals, exorbitant admission of chronic alcohol, infections and autoimmune disorders. Hepatic damage by over dose of medication seems, by all accounts, to be a typical contributing element. Liver is required to carry out physiological capacities as well as to ensure against the dangerous of unsafe medications and chemicals. Medication instigated substance harm is in charge of 5% of every healing center affirmation and 50% of all intense liver failure. More than 75% of incidents of particular medication responses bring about liver transplantation or death\(^2\).

**Pathophysiological Mechanisms**

Pathophysiological mechanisms of hepatotoxicity are still being discovered and comprise both hepatocellular/extracellular mechanisms.

**Disruption of hepatocyte:** Medications can bound to intracellular proteins by covalent tying which bring about a lessening in ATP levels prompting actin interruption. Part of actin fibrils at the surface of the hepatocyte causes blebs and burst of the layer.

**Disruption of transport protein:**

Bile stream may be interrupted by medications that influence transport proteins at canalicular film. Loss of villous procedures and intrusion of transport pumps, for example, multidrug resistance-related protein 3 forestall discharge of bilirubin bringing about cholestasis

**Cytolytic T-cell activation:** Co-valent tying of medication to Cytochrom P-450 enzyme goes about as an immunogen activating T-cells and cytokines and animating multifaceted immune reactions.
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Apoptosis of hepatocytes: Enhancement of apoptotic pathways by tumor necrosis factor-alpha receptor of Fas may trigger the course of intercellular caspases, which bring about customized cell death.

Mitochondrial disruption: A few medications restrain mitochondrial capacity by double impact on both beta-oxidation energy productions by hindering the synthesis of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, bringing about diminished ATP generation.

Bile duct injury: Dangerous metabolites disposed of in bile may bring about harm to bile conduit epithelium.

Drug induced hepatotoxicity

Medication/drug prompted liver damage is a well-being issue, and is relied upon to increment as the quantity of medications being devoured increments, both prescription and non-prescription, and because of the present pattern of utilization of pharmacologically active substances in complementary and alternative medicine. Medication/drug prompted hepatotoxicity is the most well-known reason referred for withdrawal of officially approved medications from the business. It additionally represents more than 50 percent of instances of intense liver failure in the United States. The definite frequency of medication/drug prompted liver damage is hard to gauge, and all in all, studies going to measuring its occurrence experience the ill effects of disadvantages, for example, under-reporting and that information by large originate from review studies. Regularly, there is likewise an absence of data about self-solution and utilization of herbal product that may interact with medicine and non-physician endorsed medications.

Notwithstanding the recurrence of medication instigated liver damage being low, information from the Centers for Disease Control and Prevention in the U.S. report more or less 1600 new intense instances of liver failure yearly, of which Paracetamol hepatotoxicity represents give or take 41%. At the point when taking a gender at hospitalized patients, the rate of antagonistic medication responses is evaluated to be 6.7%, and lethal unfriendly medication responses add up to 0.32%, as controlled by a meta-analysis of around 40 prospective studies. During the period 1995 to 2005, the
reports of unfavorable medication responses and additionally deaths identified with these, have dramatically multiplied. Numerous instances of medication triggered liver damage are idiosyncratic, i.e. the response is capricious taking into account the known pharmacological properties of the drug, and henceforth is barely noticeable during preclinical phases of improvement. There are however studies to show that these responses may be subject to an expanded affectability of the patient to the medication being referred to, contingent upon such components as other accompanying infections or other corresponding medications. Certain hereditary variables, for example, HLA-type, can now and again add to the affectability of a person to antagonistic medication responses. Ordinarily, clinically clear unfriendly medication responses happen when some time of idleness, anywhere in the compass going from one to 12 months (most generally inside of 90 days), and about dependably vanish after evacuation of the medication. Medication investigated liver harm may give a few distinctive clinical components; hepatitic/hepatocellular, cholestatic or mixed.

Despite their etiology, medication/drug induced hepatotoxicity remains a noteworthy issue during medication development in the pharmaceutical industry, both concerning expanded danger for patients experiencing clinical trials, furthermore patient-risk after the introduction of new medication to the treatment. Additionally, due to the expanded expenses that take after failure of a medication to-be at a late stage in medication development or after its launch.

**DRUG TOXICITY MECHANISMS**

Typical division of medication reactions is of not less than 2 noteworthy gatherings which include:

- Drugs which straightforwardly influence liver.
- Drugs which intercede an immune reaction.

**Intrinsic / predictable drug reactions:** Drugs that belongs into this classification cause reproducible wounds in animals and harm is identified with dose. Harm can be because of medication itself or to its metabolite. Acetaminophen is the most appropriate illustration of a known natural or unsurprising hepatotoxin at supertherapeutic dosages. Another illustration is carbon tetrachloride.
Idiosyncratic/unpredictable drug reactions: These drug responses can be segmented into those that are classified as hypersensitivity or immunoallergic and those that are metabolic-idiosyncratic. It happens without obvious dose-dependency and in an unpredictable fashion.
Hepatotoxicity largely indicates the chemical compelled liver destruction. Some medications when consumed in overdose and occasionally even when taken within recommend dose may damage many internal organs. Few compound/substances comprising those that are used in laboratories (Example: CCl₄ and Paracetamol) and industries (Lead, and arsenic) and natural compounds (microcystine and aflatoxins)

Figure 1.3: Three-step mechanistic working model of hepatotoxicity
and herbal therapies (cascara sagrada, ephedra) can also root hepatotoxicity. Chemicals/Compounds that cause liver damage are together branded as hepatotoxins.

- NSAIDS (Acetaminophen, Aspirin, Ibuprofen)
- Glucocorticoids.
- Anti-Tubercular drug (Isoniazid).
- Industrial toxins (arsenic, carbon tetrachloride, vinyl chloride).
- Herbal remedies (Ackee fruit, camphor, cycasin, kava leaves, valerian, comfrey).

### Alcohol Hepatotoxicity

Alcohol is one of the fundamental inducer of end-stage liver damage around the world. In the United States, alcoholic liver disease is the second most regular purpose behind liver transplantation. The Dionysos Study, a cohort investigation of the predominance of ceaseless liver disease in an Italian populace, demonstrated that 21% of the populace considered was at danger for creating liver damage. Of these, just 5.5% of the people at danger hinted at real liver damage. Around 50 years prior it was accepted that alcohol in itself was not harmful, rather that the dietary inadequacies frequently going hand in hand with it were the real reasons for liver harm. In any case, it was indicated by Lieber and De Carli that in rats, alcoholic liver damage created in spite of adequate sustenance. The lethality of alcohol was later on demonstrated to be identified with its digestion system by alcohol dehydrogenases (ADHs) furthermore to the digestion system by CYP2E1. There is additionally a part of digestion system by catalase. The fundamental pathway for ethanol (EtOH) oxidation in the liver is by means of ADH to acetaldehyde, which is connected with the reduction of NAD to NADH. NADH thus builds xanthine oxidase action, which rises generation of superoxide. Metabolic system of EtOH by alcohol dehydrogenase impacts the redox status of the liver likewise in different ways. Elevated acetaldehyde creation after EtOH metabolism diminishes hepatic glutathione (GSH) content. The diminishing in GSH is both because of an expanded misfortune, and also a lower rate of synthesis.
Ethanol induces number of deleterious metabolic changes in liver. Intake of ethanol for long time leads to development of steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes. About 80% of heavy drinkers had been
reported to develop steatosis, 10-35% alcoholic hepatitis and approximately 10% liver cirrhosis\textsuperscript{7}.

**Mechanism underlying Ethanol induced hepatotoxicity**

Alcohol consumption results in increase in release of *endotoxin* from gut bacteria and membrane permeability of gut to endotoxin or both. Females are more often sensitive to these changes. Blood endotoxin is elevated and enters liver where it is engulfed by Kupffer cells that become activated releasing TNF- alpha, PGE2 and free radical. Prostaglandins increase oxygen uptake and are responsible for hypermetabolic state in liver. Increase in oxygen demand leads to hypoxia of liver and on reperfusion alpha-hydroxyethyl free radicals are formed that leads to tissue damage in oxygen poor pericentral regions of liver lobule. Blocking of these events can be done by sterilization of gut using antibiotics or destruction of Kupffer cells with Gdcl3 and thus prevents liver injury\textsuperscript{8}.

![Figure 1.6: Mechanism of ethanol induced hepatotoxicity](image)

**Symptoms of Hepatotoxicity**

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**Figure 1.6: Mechanism of ethanol induced hepatotoxicity**
List of signs and symptoms depicted in various causes for Hepatotoxicity include 15 symptoms as listed below:

- Nausea
- Vomiting
- Abdominal pain
- Loss of appetite
- Diarrhea
- Tiredness
- Weakness
- Jaundice
- Yellow eyes
- Yellow skin
- Hepatomegally
- Abnormal liver function test results
- Swelling in feet
- Weight gain due to water retention
- Prolonged bleeding time.

**Treatment for Hepatotoxicity**

The list of treatments mentioned in various sources for hepatotoxicity includes the following. Always follow professional medical advice about any treatment or change in treatment plans. Treatment of hepatotoxicity is depends upon causative agent, degree of liver dysfunction and age and general health of patient. Treatments for hepatotoxicity include:

- Withdrawal of causative medication or removal from exposure to causative agent.
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Regular monitoring of patient and review of liver function – where liver dysfunction is mild to moderate and liver function is improving.

Complete avoidance of alcohol and medication that may contribute to further liver damage.

N-Acetylcysteine is used for paracetamol toxicity.

Management of symptoms of liver damage.

- Nutrition – with vitamin supplementation as required
- Regular exercise in order to maintain muscle mass.
- Ursodeoxycholic acid.

Management of pruritus

- Cholestyramine
- Antihistamines.

Management of ascites

- Low sodium diet.
- Diuretics – furosemide, spironolactone.
- Removal of fluid via a needle in the abdomen – Paracentesis.
- Portosystemic shunting.

Management of portal hypertension

- Beta – blockers
- Oesophageal variceal banding
- Portocaval shunt

Management of acute liver failure due to hepatotoxicity

- Supportive care always in intensive care unit – airway protection, fluid and electrolyte management.
- Management of complications such as bleeding problems and hepatic encephalopathy.
Liver transplantation – for acute fulminant liver failure or end stage cirrhosis.

Modern Medicines for Treatment of Liver Diseases

Liver diseases can be treated using allopathic as well as by using herbal drugs.

Hepatoprotective Allopathic Treatment

Few modern medicines are available for treating liver diseases that includes:

- **Ursodeoxycholic acid (Ursodiol):** Ursodiol decreases intestinal absorption and suppresses hepatic synthesis and storage of cholesterol. It is mainly used in management of chronic hepatic diseases in humans.

- **Penicillamine:** Penicillamine chelates several metals like copper, iron, lead and mercury forming stable water soluble complexes which are renally excreted.

Other drugs:

Antiviral medication such as alpha interferon, ribavirin, steroids, antibiotics etc. are also used in liver diseases. Drugs like tricholinecitrate, trithioparamethoxy phenyl propane, essential phospholipids, combination of drugs such as L-ornithine, L-aspartate and pancreatin, silymarin and Ursodeoxycholic acid are usually prescribed for hepatitis, cirrhosis and other liver diseases. N-acetylcysteine is used in early phases of acetaminophen toxicity. L-carnitine is potentially valuable during valproate toxicity. Cholestyramine can be used to alleviate pruritus.

Disadvantages of allopathic drugs

Side effects of many modern medicines are mostly alarming. Interactions, contra-interactions, side effects and toxicity of synthetic medicine vary from mild to severe that includes insomnia, vomiting, fatigue, dry mouth, diarrhea, constipation, dizziness, suicidal thought, depression, seizures, anemia, hair loss, high blood sugar, swelling, impotency, confusion, fainting and finally death. Antibiotics usually cause stomach upset or allergic reactions. Interferon shows side effects as flu-like illness with fever and body aches.

Herbal Hepatoprotective Drug Treatment
A number of polyherbal preparations have been used in treating various liver disorders since ages. Some herbal formulations include:

a) Liv-52: It is a non-toxic hepatoprotective drug from Himalaya Drug Co. Liv-52 can improve clinical parameters in patients having liver damage mainly in alcoholic liver cirrhosis.

b) LIMARIN®: It has potent hepatoprotective and free radical scavenging (antioxidant) activity. It is derived from active extract of fruit of silybum marianum.

Some of the polyherbal formulations have been evidenced for hepatoprotective activity against chemical driven liver damage in experimental animals which include Liv52, Liv42, Jigrine, Koflet, Cirrhitin, Livex and Hepatomed etc.

**Problem In Hand**

**Limitations of herbal preparations**

Herbal-based preparations for treating liver disorders has been used in India for long time and has been popularized worldwide by leading pharmaceuticals. Despite of popularity of herbal medicines for liver diseases in particular, are still unacceptable treatment modalities for liver diseases. Limiting factors include:

- Lack of standardization procedures of herbal preparations.
- Lack of identification of active components and principles.
- Lack of randomized controlled clinical trials (RCTs).
- Lack of toxicological evaluation.
- Poor solubility.
- Poor bioavailability.
- Poor hepatic cell regeneration.

**Hepatoprotective Mono-Herbal Medicines**
Medicinal plants are significant sources of hepatoprotective drugs. Almost 160 phytoconstituents from 101 plants have been claimed by Pharmacopoeia Foundation to possess hepatoprotective action\textsuperscript{11}. Herbal drugs are most widely used than allopathic drugs as hepatoprotectives because these are usually inexpensive, better cultural acceptability, improved compatibility with human body and minimal side effects. Various classes of phytoconstituents like flavonoids, triterpenes, lignans, steroids, glycosides, polyphenols, saponins, coumarins and volatile oils etc posses hepatoprotective activity.

**Flavonoids**

Among numerous plant-based bioactive compounds, flavonoids have been studied extensively and have shown promising results improving various disease aspects. Flavonoids belong to the comprehensive category of polyphenols and six classes, namely flavan-3-ols (including proanthocyanidins), flavonols, anthocyanins, isoflavones, flavanones and flavones are found in plant sources.

Flavonoids exert beneficial effects on health directly and/or indirectly. Previous literature shows evidence that flavonoids (proanthocyanidins as an example) are antioxidants and hypocholesterolemic compounds as well as modulators of cell signaling and gene expression in different experimental models and in human epidemiological studies\textsuperscript{68}.

Considering the distribution of flavonoids, some classes can be found in many foods whereas the presence of some classes is limited to certain foods. As an example, flavonols can be commonly found in fruits, vegetables and teas; however, isoflavones are almost exclusively located in leguminous plants.

Dietary sources of flavonoids and their intake can vary widely among populations as it depends on the availability of dietary sources, dietary practices and food habits of different demographic groups.

Flavonoids are natural products widely distributed in plant kingdom. Flavonoids are capable of modulating activity of enzymes and affect behavior of several cell systems and exerting beneficial effects on body. Several flavonoids such as Quercetin, Rutin, Silymarin etc are reported for their hepatoprotective activities.
### Rutin

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>2- (3,4 - di hydroxy phenyl)- 5,7-di hydroxy-3-[α-L - rhamno pyranosyl-(1→6)-β-D -gluco pyranosyl oxy]-4H-chromen - 4 - one</th>
</tr>
</thead>
</table>
| Organoleptic Characterization | Appearance : Yellow to green powder  
Mel Pt: 195 Degrees Celcius  
Solubility : Unable to H2O--soluble |
| Empirical Formula | C\textsubscript{27}H\textsubscript{30}O\textsubscript{16} |
| Pharmacokinetic Parameter | Rutin  
\(C_{\text{max}}\) (ng/ml) | 53- 152  
\(T_{\text{maxβ}}\) (hr) | 1.9-2.2  
\(T \frac{1}{2}\) (hr) | 17.1-20.2  
\(\text{CL}_R\) (l/hr) | 0.06-6.89  
AUC (ng/ml hr) | 702-1473 |
| Pharmacological Activity | Rutin posses the following pharmacological activity free radical scavenging activity, diabetic activity, peptic ulcer, hepatoprotective & chemoprevention of cancer. |
| Side Effects | Headache, flushing, stomach upsetβ & rashes. |
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<table>
<thead>
<tr>
<th>Drug Interaction</th>
<th>✓ Antibiotic Quinolones like ciprofloxacin, levofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>✓ Cyclosporine conc. is elevated when co administered with Rutin</td>
</tr>
<tr>
<td></td>
<td>✓ Rutin should not given along with antihypertensive drugs like enalapril, captopril, valsartan, amlodipine, hydrochlorothiazide &amp; furosemide because quercetin tensed to reduced the Blood Pressure</td>
</tr>
</tbody>
</table>

| Contra-indications | Caring & breast-feeding, women |

Rutin is a bioflavonoid. It is a yellow crystalline flavon glycoside (C27H30O16) which occurs in a variety of plants (rue, tobacco, buckwheat etc.). On hydrolysis rutin yields quercetin and rutinose (disaccharide).

![Figure 1.7: Structure of Rutin](image)

Rutin is a naturally occurring flavonol consisting of aglycone quercetin and a rutinoside moiety in position 3 of c ring. Pharmacological activity of rutin and its aglycone may differ and presence of rutinoside moiety is responsible for some of the protective effects of rutin. Rutin exhibit multiple pharmacological activities including antibacterial, antitumor, antiviral, antiprotozoal, anti-inflammatory, antiallergic, antiplatelets activity. Also have antiulcer, antidiarrhoeal, antispasmodic, myocardial protecting, antimutagenesis, vasodilator activities. Rutin stabilizes plasma membrane and also increases regenerative potential of liver.
Hepatoprotective Activity of Rutin

Rutin can ameliorate acute liver damage by at least four mechanisms which include acting as scavengers of free radicals, inhibiting NF-Kb activation and inflammatory response exerting antifibrotic potential and inducing Nrf2/HO-1 pathway. Rutinoside moiety in position 3 of c ring is responsible for more pronounced protective effects against hepatocellular necrosis. Rutin exerts stronger protection against nitrosative stress and hepatocellular damage.\(^\text{13}\)

Anticancer Activity of Rutin

Rutin induces cell cycle arrest and apoptosis in murine leukemia (WEHI-3) cells in vitro and in vivo. Rutin has antimutagenic action.\(^\text{14}\) It is an inhibitor of carcinogenic processes and is also a potential cancer chemo preventive agent. Rutin has modulating effects on mutagenic anticancer drug Mitomycin C by single cell gel electrophoresis in human lymphocytes. In human lymphocytes rutin displayed protective effect on DNA damage induced by Mitomycin C in concentration dependent manner.\(^\text{15}\)

Limitations and side effects of rutin

- Rutin when administered orally shows poor absorption because of less lipophilicity.
- Rutin is poorly water soluble (12.5mg/100ml of water).
- Rutin is light sensitive compound so should be encapsulated inorder to enhance its solubility.

Rutin is unsafe when taken in very high dose for long periods of time. It can cause some side effects such as headache, flushing, skin rashes or gastric discomfort.

Quercetin

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>3 , 3', 4', 5 , 7- penta hydroxy flavone</th>
</tr>
</thead>
<tbody>
<tr>
<td>β Organoleptic Characterization</td>
<td>Forms : Yellow-crystalline materials</td>
</tr>
<tr>
<td></td>
<td>Mel Pt: 316° degree celcius</td>
</tr>
<tr>
<td>Chapter 1</td>
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<tr>
<td><strong>Introduction</strong></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Empirical Formula</th>
<th>Solubility : Insoluble in H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{15} H_{10} O_{7}</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Quercetin aglycone</th>
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</thead>
<tbody>
<tr>
<td>C_{\text{max}} (ng/ml)</td>
<td>0.49–39.9</td>
</tr>
<tr>
<td>T_{\text{max}} (hr)</td>
<td>1–5</td>
</tr>
<tr>
<td>T^{1/2} (hr)</td>
<td>0.956–12.5</td>
</tr>
<tr>
<td>CL_{R} (l/hr)</td>
<td>0.06–6.89</td>
</tr>
<tr>
<td>AUC (ng/ml hr)</td>
<td>2.25–182</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Quercetin Metabolites</th>
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</thead>
<tbody>
<tr>
<td>C_{\text{max}} (ng/ml)</td>
</tr>
<tr>
<td>T_{\text{max}} (hr)</td>
</tr>
<tr>
<td>T^{1/2} (hr)</td>
</tr>
<tr>
<td>CL_{R} (l/hr)</td>
</tr>
<tr>
<td>AUC (ng/ml hr)</td>
</tr>
</tbody>
</table>

| Pharmacological Activity | Quercetin withholds the subsequent pharmacological commotion free radical scavenging activity, anti atherosclerotic effect, diabetic activity, peptic ulcer, hepatoprotective & prevention of cancer. |
## Drug Interaction

<table>
<thead>
<tr>
<th>Drug Interaction</th>
<th>Antibiotic Quinolones(\beta) like ciprofloxacin, levofloxacin, Gatifloxacin ofloxacin.</th>
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<tr>
<td></td>
<td>Cyclosporine conc. is elevated when co administered with Quercetin.</td>
</tr>
<tr>
<td></td>
<td>Quercetin should not given along with antihypertensive drugs like losartan, enalapril, captopril, valsartan, amlodipine(\beta), hydrochlorothiazide &amp; furosemide because quercetin tensed to reduced the Blood Pressure.</td>
</tr>
<tr>
<td></td>
<td>Quercetin might prevent Pgp transport mechanism due to this processes some drugs Paclitaxel, dilidizam, vincristine, vinblastin, ketoconazole (\beta)(\beta).</td>
</tr>
</tbody>
</table>

### Contraindication

- Pregnant & Lactating women

Quercetin (3, 3', 4', 5, 7- pentahydroxyflavone) is one of the most abundant flavonoids and is widely distributed in nature\(^\text{16}\). Quercetin (name comes from Latin quercetum meaning oak forest, quercus oak) is abundant in apples, berries, broccoli and onions. Quercetin consists of 3 rings and 5 hydroxyl groups.

![Figure 1.8: Structure of Quercetin](image-url)

Figure 1.8: Structure of Quercetin
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It is found in most plants, fruits and vegetables which can reach levels in human diet as high as 16-25mg/day. It shows therapeutic potential against various liver injuries caused by toxins which include CCl₄, ethanol, paracetamol, thioacetamide etc\(^{17}\).

Quercetin contains number of phenolic hydroxyl groups and is a potent oxygen free radical scavenger and a metal chelator\(^{18}\). Anti-proliferative potency of quercetin would be attributed to hydroxyl substitutions particularly hydroxyl- substitutions at carbon 3 of ring B and carbon 5 of ring A.

**Hepatoprotective Activity of Quercetin**

Quercetin is a P-gp inhibitor reverse P-gp- mediated efflux and thus improves efficiency of drug transport across epithelia and also inhibit some of enzymes involved in drug metabolism, increases bioavailability, blood levels and efficacy of number of drugs.\(^{19}\)

**Anticancer Activity of Quercetin**

Quercetin is reported to inhibit cytochrome P450 enzymes of CYP₁A family. Quercetin also inhibits CYP₃A₄ which is most abundant CYP P450 enzyme in liver and beneficial in metabolizing a significant number of carcinogens and medications. Quercetin inhibits protein tyrosine kinase which is also involved in cell proliferation. Quercetin inhibits proliferation and increases osteogenic differentiation in human adipose stromal cells. Quercetin possesses strong anti-tumor activity via a reactive oxygen species (ROS) dependent apoptosis pathway.

**Limitations of Quercetin**

- Quercetin is a good antioxidant but with limited clinical application because of its hydrophobic nature and limited bioavailability.
- Poor absorption and extremely low distribution to brain after oral administration due to both rapid metabolism and difficulties in penetration through blood brain barrier.
- Poor water solubility
# SILIBININ

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>2,3 ( R )-((2 R,3 R)-3,5,7)-trihydroxy (-2-{(2 R,3 R)-3-(4\text{-hydroxy -3-methoxy phenyl})-2\text{-(hydroxy methyl) -2,3-dihydro benzo}[b][1,4]\text{di oxin-6-yl]} \text{ chroman -4-one} )</th>
</tr>
</thead>
</table>
| Organoleptic Characterization | Appearance : pale white 
Melting Point: 167° C 
Solubility: Insoluble in H2O |
| Empirical Formula | C\(_{25}\) H\(_{22}\) O\(_{10}\) |
| Pharmacokinetic Parameter | Silibinin 
\( C_{max} \text{ (ng/ml)} \) 23-61 
\( T_{maxβ} \text{ (hr)} \) 0.5-2 
\( T \frac{1}{2} \text{ (hr)} \) 0.6-1.6 
\( CL_R \text{ (l/hr)} \) 970 
\( AUC \text{ (ng/ml hr)} \) 118-557 |
| Pharmacological Activity | ✓ Silibinin might protect Hepatic cells from toxic chemicals & drugs Acetaminophen, Carbon tetrachloride & Ethyl alcohol. 
✓ It in addition seem antioxidants & anti-inflammatory effects. Silibinin plant extract might enhance the effects of estrogen. |
| Side Effects | ✓ Nausea, diarrhea, indigestion, bloating, fulness or ache, & non-apetite. 
✓ It can cause an allergic reaction in people perceptive Asteraceae/ folks including ragweed, mari-golds, , & further interrelated vegetation. |
Drug Interaction

- **Cytochrome P450 2C9 (CYP2C9) substrates**
  - amitriptyline, diazepam, zileuton, celecoxib, diclofenac, glipizide fluvastatin, profen, ir-besartan, losartans, β piroxicams, tolbutamides, torsemides, warfarin, phenytoin
- Sirolimus, HMG-coAReductase Inhibitors
  - Fluvastatin, Atorvastatins, β Lovastatin, Pravastatin β Rosuvastatins
- Tamoxifen.

Contraindication

- Pregnant or breast-feeding.
- Sensitive or sensitive to to rag-weeds, mari-golds, & other related plants.
- Hormone-sensitive condition. Few of the circumstances comprises endo-metriosis; endo-uterine cyst; & malignoma of the mammary, β & ovaries.

Silibinin is a major active constituent of silymarin. Milk thistle (Silybum marianum) is a part of Asteraceae family and is one of the most ancient herbal medicines. Seeds of milk thistle have been used for more than 2000 years to treat liver diseases. Medicinal value of milk thistle seeds is due to silymarin. Silymarin is a potent hepatoprotective drug with established place in hepatology practice. Silymarin is a mixture of Silybin (silibinin), isosilybin, silychristin and silydianin. But silibinin is the component with greatest degree of biological activity and comprises of 50-70% of silymarin.  

**Hepatoprotective Activity of Silibinin**

Mechanism of action of Silybin is complex and highly beneficial in protecting hepatocytes. It blocks penetration of various toxins into hepatocytes and thus prevents cell death. It protects liver from oxidative intracellular free radicals by increasing activity of enzyme superoxide dismutase and peroxidase as well as by increasing
concentration of glutathione and activity of peroxidase. Silybin strengthens and stabilizes cell membranes, inhibits synthesis of prostaglandins associated with lipid peroxidation and promotes regeneration of liver through stimulation of protein synthesis and thus effects on production of new hepatocytes. Silybin acts in four different ways:

- **Antioxidant, scavenger and regulator of intracellular content of glutathione.**
- **Cell membrane stabilizer and permeability regulator that prevent hepatotoxic agents from entering hepatocytes.**
- **Promoters of ribosomal RNA production, stimulating liver regulation.**
- **Inhibitors of transformation of stellate hepatocytes into myofibroblasts - process which is responsible for deposition of collagen fibers leading to cirrhosis**

![Structure of Silibinin](image)

**Figure 1.9: Structure of Silibinin**

**Anticancer Activity of Silibinin**

Silibinin mainly induces growth inhibition, moderate cell cycle arrest and strong apoptotic death in both small cell and non-small cell human lung carcinoma cells. Silibinin has pleiotropic anticancer effects on prostate cancer (PCA) cells leading to cell growth inhibition. Mechanisms of silibinin efficacy against PCA involve alteration in cell cycle progression and inhibition of mitogenic and cell survival signaling like epidermal growth factor receptor, insulin like growth factor receptor type 1 and nuclear factor Kappa B signaling. Silibinin synergizes therapeutic property of doxorubicin in PCA cells making it strong candidate for combination chemotherapy.
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Introduction

Limitations of Silibinin: It is hydrophobic in nature so results in low bioavailability, poor absorption, rapid metabolism and poor oral bioavailability.\(^{22}\)

Although flavonoids have shown countless health benefits, their low bioavailability has been a concern. Phase 2 metabolism is known to affect the bioavailability of flavonoids in humans. Usually, most flavonoids undergo sulfation, methylation and glucuronidation in the small intestine and liver and conjugated metabolites can be found in plasma after flavonoid ingestion. In general, metabolites of flavonoids show reduced bioactivity in comparison to parent compounds but there have been results that reported otherwise as well. Despite the bioactivity expressed in different in vitro systems, bioavailability of flavonoids would be a determinant factor of their bioactivity in vivo. Therefore, enhancement of bioavailability would be of utmost importance in order to exert health benefits, in vivo. In this regard, numerous attempts have been made to increase bioavailability such as: improving the intestinal absorption via use of absorption enhancers, novel drug delivery systems; improving metabolic stability; changing the site of absorption from large intestine to small intestine. This review briefly discusses: flavonoids belonging to the six classes, some of the factors that determine their bioavailability, recent studies attempting to improve their bioavailability that in turn can improve their bioactivity in vivo.

Novel approaches in overcoming the limitations of conventional drugs

Various approaches have been reported to overcome the limitation of conventional drugs including (a) Crystal modifications (which includes metastable polymorphs, salt formation and co-crystal formation); (b) Amorphization (which includes cyclodextrin complex, self-emulsification and pH modification); and (c) Particle size reduction (which includes micronization and nanotechnology). Of all, nanotechnology is the most recent and showing encouraging results.\(^{23,24}\)

Nanotechnology

Nanotechnology is a science that deals with engineering particles on a near atomic and molecular scale with at least one dimension in the range of 1-1000 nanometers (nm). Size reduction in the nano range significantly enhance the surface area and reactivity, which modify the physicochemical properties of the size reduced
compound and offer significant improvement in various fields of sciences including automotive, electronics, energy and medicine.\textsuperscript{23,25}

**History of nanotechnology\textsuperscript{25}**

One of the most documented examples of nanotechnology known in history was the medieval stained glass artisans. They were the first, although unaware, trapped gold nanoparticles in the ‘glass matrix’ in order to generate the ruby red colour in the windows. They also trapped silver nanoparticles, which gave a deep yellow colour. As in today's finding it was the size of the metal nanoparticles, define variations in colour.

Deruta ceramicists are the another example of the practice of the early forms of nanotechnology. The people in Umbria, Italy (1450-1600AD) used nanotechnology to produce iridescent or metallic glazes. They achieved these effects by using particles of copper and silver metal (between 5 and 100 billionth of a meter) in their glazes, this caused light to bounce off their surface at different wavelengths, thus giving it the ‘iridescent’ look.
The Lycurgus Cup was made by the Romans at around the 4th century (AD). An interesting factor about the cup was that the colour of the cup can change. When it is looked at in reflected light or daylight, it appears green. However, when light was shone into the cup and transmitted through the glass, it changes the colour to red. The unusual optical properties are due to the glass containing tiny amounts of colloidal gold and silver. Colloidal gold is nanoparticles of pure gold suspended in water.

In 1959, the great physicist Richard Feynman proposed the possibility of manoeuvring things atom by atom in his lecture entitled “Plenty of Room at the Bottom” at the California Institute of Technology.

In 1974, Japanese scientist Norio Taniguchi of the Tokyo University of Science invented the term nanotechnology.

In 1977, K. Eric Drexler developed and popularized the concept of nanotechnology and founded the field of molecular nanotechnology and written the first paper on advanced nanotechnology.

In 1980s, various kinds of nanoparticle systems were made, which includes stable, dispersible materials of almost every element and common binary oxides and sulphides.
In 1981, Gerd Binnig and Heinrich Rohrer developed scanning tunnel microscope, an instrument for imaging surfaces at the atomic level.


In 1983, self-assembled monolayer (molecularly thin film) of thiols on gold surfaces was made. These are used for various nanopatterning applications.


In 1986, Binnig, Calvin Quate and Christopher Gerber invented the first atomic force microscope.

Chapter 1
Introduction

In 1986, the first book on nanotechnology entitled “Engines of Creation: The Coming Era of Nanotechnology” was published by K. Eric Drexler, which provocative ideas on molecular nanotechnology to a general audience.


In 2003, a micro-organism, *Rhodococcus sp.*, which normally grows on fig trees, has been used to synthesize gold nanoparticles.

In 2004, world’s first college of nanotechnology was established at Suny Albany, USA.

In 2005, the concentration of amyloid-β-diffusible ligands in cerebrospinal fluid has been measured using a nanoparticle-based bio-barcode assay. This technique could provide a method for the early diagnosis of Alzheimer’s disease.
In 2005, an atomic force microscope that works in liquid has been made, which could be used for imaging biological samples, easily oxidizable materials and samples in hazardous environments.

In 2005, poly(ethylene glycol) modified gelatin nanoparticles have been tested as gene delivery vehicle for tumor specific cells employing both in-vitro and in-vivo studies. Such a biocompatible system was highly desirable for targeted and controlled delivery of genetic constructs to solid tumors.

In 2005, Quantum dots modified with DNA probes have been used to detect DNA targets using fluorescence resonance energy transfer. The specificity of this technique was unparalleled, while the detection capabilities of single-point mutation have been reported.

In 2005, magnetic, fluorescent nanoparticles functionalized with a variety of biological molecules have been used to differentiate endothelial cells. This material is used for in-vivo cancer imaging in the pancreas. This could also find application in differentiating cell lines, exploring cellular states and targeting specific cell types.

**Nanomedicine**

Nanotechnology in medicine is referred as ‘Nanomedicine’ and it provides significant development in diagnosis and treatment of various disorders via nanoparticulate drug delivery system, which includes solid-lipid nanoparticles, metallic nanoparticles, nanocrystals, nanosponges, liposomes, magnetic nanoparticles, protein-based nanoparticles, hydrogel nanoparticles, dendrimers, fullerene nanoparticles, superparamagnetic nanoparticles, ceramic nanoparticles, carbon based nanomaterials, polymeric nanoparticles and polymeric micelles. In general, nanoparticulate drug delivery system provides sizeable advantages which includes \(^{23,25-31}\)
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### Introduction

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**In vivo Imaging Segment**

| Resovist | Iron Nanoparticles | Liver tumor | Schering |

**In vitro Diagnostics**

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SCOPE OF RESEARCH WORK:

☐ Enhancement of aqueous solubility of hydrophobic drug.
☐ Protection of encapsulated drug from the degradation.
☐ Improvement in the bio-distribution and circulation time of the drug.
☐ Provides control and sustain release of the drug.
☐ Increases the intracellular concentration of drug.
☐ Reduces the number of required doses.
☐ Provides an opportunity to incorporate both hydrophilic and hydrophobic drugs in a single polymeric matrix.
☐ Feasibility of administration through various routes including oral, nasal, parenteral, intraocular etc.
☐ Reduces the systemic toxicities of the drug by encapsulation and targeting the drug to specific site.
☐ Helps to regenerate the hepatic cell

Similarly, various nanoparticulate drug delivery systems to overcome the limitations of Quercetin, Rutin and Silibinin have been reported, which were summarized in chapter 2 (Literature Review).