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Herbal drugs as Hepatoprotective Agents

Plants have been used therapeutically since time immemorial in a variety of conditions. They play a key role in the human health care. The traditional medicine refers to a broad range of ancient, natural health care practices including folk /tribal practices as well as Ayurveda, Siddha, Amchi and Unani (Subramonium, 1999).

In contrast to the narrow spectrum of activity of synthetic drugs with its attendant risk of side effects, herbal drugs as traditionally used are obtained by processing of material by procedures such as drying, powdering, extraction either using heat or at room temperature using water, etc. to give products which represent the totality of the material and which are mixtures of several components. Both the pharmacological profile of the components and the chemical classes to which they belong are complex and the activity shown by such mixtures is also broad.

There has been historically deep scepticism of doctors trained in modern medicine that herbal drugs could be really doing anything more than serving as a placebo or a palliative and in the case of drugs for liver disorders perhaps increasing the flow of bile. However, in recent times there has been a large volume of work aimed at scientific validation of the efficacy of herbal drugs, including those classified to be hepatoprotective, coinciding with the world-wide popular perception of herbal drugs as safe and effective alternatives or adjuncts to modern medicine and their

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iatrogenic effects. This interest in herbal drugs also stems from the fact that modern medicine does not have a suitable answer for many conditions such as arthritis, asthma and many skin conditions etc.

In the traditional system of medicine various drugs have been used in the treatment of liver diseases. Some of these include:

Andrographis paniculata, Berberis aristata, Boerhaavia diffusa, Cichorium intybus, Citrollus colocynthis, Cocculus vilosus, Eclipta alba, Embelia ribes, Fumaria officinalis, Indigofera tinctoria, Melia azadirachta, Mimosa pudica, Picrorrhiza kurroa, Piper nigrum, Phyllanthus niruri, Podophyllum emodi, Solanum nigrum, Swertia chirata, Terminalia chebula, Tinospora cardifolia etc.

The drugs used for treatment of jaundice in the Indigenous system of medicine include:

Boerhaavia ripens, Citrollus colocynthis, Melia azadirachta, Tinospora cardifolia, etc.

The Indigenous drugs have great importance both from the professional and economic point of view. A large number of these plants and plant products have been reported to possess choleretic, cholagogue or antihepatotoxic activities (Doroswamy, 1995).
SOME COMMONLY USED PLANTS IN HERBAL FORMULATIONS
FOR LIVER DISORDERS

*Andrographis paniculata (28)*
*Boerhaavia diffusa (10)*
*Eclipta alba (10)*
*Picrorrhiza kurroa (10)*
*Oledenlandia corymbasa (10)*
*Astercantha longifolia (8)*
*Apium graveolens (8)*
*Cassia occidentals (8)*
*Cichorium intybus (8)*
*Embelia ribes (8)*
*Tinospora cardifolia (8)*
*Trachyspermum ammi (8)*

Scientifically validated in experimental animals
* Each plant is used in more than the number of formulations given in bracket. (Ref: Subramanian 1999)

Increased secretion of bile and bile substances by direct action on liver cells was caused by onions, *Allium cepa* (Liliaceae) (Schindel, 1934). Different concentrations of polar fractions of garlic, *A sativum*, have been reported to cause dose related decrease in the activities of hepatic beta-hydroxy-beta-methylgluteryl Co-A reductase, cholesterol, 7-alpha-hydroxy base and fatty acid synthetase (Quireshi, 1983).

Extracts of *Aloe* (Liliaceae) when given i.v, increased the flow of bile in the anaesthetized dogs (Hazleton, 1942).
Pretreatment of rats with ethanolic extract from the leaves of *Acuba japonica* (Cornaceae) protected against *CCl₄* induced depression in plasma disappearance and biliary excretion (Yang, 1983).

Aqueous and acetone extracts of *Boerhaavia diffusa* (Nyctaginaceae) inhibited the SGOT and SGPT and increased the liver ATP-ase activity in albino rats (Bhalla et al., 1968).

Infusion of *Calendula officinalis* (Compositae) has shown a pronounced choleretic effect in dogs (Naumenko, 1941).

In African folklore medicines ‘Kinkeliba’ botanically identified as *Combretum micranthum* (Combretaceae), has been used for liver disorders and is reported to possess cholagogue properties (Decaus F, 1948).

*Daucus carota* (Umbelliferae) fed rats have been found to show increased resistance to *CCl₄* induced hepatotoxicity (Forbes J.C, 1945).

Oral administration of aqueous extract of *Delphinium denudatum* (Ranunculaceae) to rats prior to *CCl₄* treatment, antagonized the effect on *CCl₄* by significantly elevating the levels of liver glycogen and adrenal ascorbic acid and depleting the levels of other metabolites, thus showing its protective effect on the liver against *CCl₄* induced hepatotoxicity (Khan A. 1981).

Hepatic necrosis in rats, caused by allyl alcohol, is significantly reduced when rats are prefed orally with *Eclipta alba* (Composite) leaf juice (Joglekar, 1967). The juice given daily to rats at a dose of 0.4ml orally for a period of three months along with *CCl₄* was found to maintain normal
hepatic functions (Khin M.M, 1978). Ethanolic extract of *E alba* has also been reported to reduce the elevated serum ALT levels and centrilobular necrosis against paracetamol induced hepatocellular damage in mice (Tabassum N et.al 1997; 2004).

Alcoholic extract of *Embelia ribes* seeds has been reported to reduce the fatty deposits in liver, serum transaminases and total lipids against chronic toxicity induced by country made liquor in rats (Tabassum N and Aggrawal S.S 1997). It has also been reported to produce a dose dependent fall in serum ALT levels in the acute model using paracetamol as hepatotoxin in mice (Nahid et.al 2003).

The elevation of SGPT and accumulation of lipids in the liver of mice induced by CCl₄ was antagonized by an alcoholic extract of *Ganoderma lucidum* and *G japonicum* (Polyporaceae) (Liu G.T, et.al. 1980).

Alcoholic extract of the aerial parts of the plant *Indigofera tinctoria* (Leguminosae) has exhibited antihepatotoxic effect against CCl₄ induced hepatic injury in rabbits, rats and mice (Anand K, 1979; 1981).

The tincture of *Myrica cerifera* (Myricaceae) increased the volume of bile in a clinical trial on 14 patients at a dose of 10 drops twice daily for a period as long as 70 days (Boeriche G.W, 1942).

Petroleum ether extract of the seeds of *Nymphaea stellata* (Nymphaeaceae) reduced sleep time prolongation and significantly prevented increase in liver weight and volume and also prevented hepatic necrosis induced by CCl₄ in mice and rats (Singh N 1978).
*Piper longum* (Piperaceae) improved regenerative process by restricting fibrosis in rats against CCl₄ induced hepatotoxicity (Nirmala, R 1984).

Intravenous injection of resin of *Podophyllum* (Beriberidaceae) increases the flow of hepatic bile in the anaesthetized dog (Hazleton I.W 1942).

Dried 90% alcoholic extract prepared from the fruits of *Terminalia belerica* (Combretaceae) produced increase in bile flow with an increase in solid contents, when administered to dogs (Siddiqui H.H, 1963).

The heartwood as well as sapwood extract of lime wood *Tilia* (Tiliaceae) showed choleretic properties (Orsymonde, S A 1963). The aqueous extract of the defatted bark of *T platyphylllos* exhibited fairly strong and prolonged choleretic activity in rabbits (Gorka, Z 1966).

*Tinospora cardifolia* (Menispermaceae) has been proved to be effective in preventing fibrous changes and promoting regeneration of liver parrenchymal tissue against liver injury by CCl₄ (Nirmala R 1984).

The alcoholic extract of the defatted seeds of *Withania somnifera* (Solanaceae) has been found to reduce sleep time prolongation and prevent increase in liver weight and volume and also prevent necrosis of liver tissues (Singh N 1978).
Some of the locally available plants in Kashmir which have been used in traditional system of medicine for treatment of liver disorders are:

*Adiantum capillus veneris* - Common name Maiden hair fern; Local name: Geowthir. Boiled in wine and given in cases of tumour of liver (Anonymous 1986).

*Achillea mellifolium* Linn. (Asteraceae): Common name Arrow root / Bloodworm; Local name: Pahel gassa/ Pahel kutch. Preliminary screening of *A mellifolium* showed antihepatotoxic activity (Gadgoli and Mishra 1995) Screening of the hepatoprotective effect of Liv-52, a polyherbal formulation, on ethanol metabolism in chronic alcohol exposed rats has also shown positive results. (Chauhan et.al 1994).

*Plantago lanceolate* Linn. (Plantaginaceae): Common name: Plantain; Local name: Gulla. Nitrite induced liver damage has been reported to be reduced by the phenolic complex plantastine in rats (Karpilovskaia et.al 1989: Inouye et.al 1974).

*Arctium lappa* Linn. (Asteraceae): Common name: Burdock; Local name: Phagorrra. Arctiin has shown protection against acetyl amino fluorine induced liver damage in rats (Kato et.al. 1998).


Berberis lycium Royle. Common name: Barberry; Local name: Kawdach. Reported to be useful in jaundice (Kaul, 1997).


Cyprus rotundus Linn: (Cyperaceae). Common name: Nut grass. Local name: Gam gassa. Methanol extract of dried rhizome at a dose of 670mg/kg showed strong activity against CCl4 induced hepatotoxicity (Chang et.al. 1984).

Fumaria indica: Common name: American fumitory. Local name: Shahtar. Dried plant reported to be liver tonic (Kaul 1997).

Iris decora Wall: Local name: Sosan. Root powder useful in bilious obstructions (Chopra, 1956).

**Ocimum sanctum**: Local name: Baber beoil. Infusions of leaf given in hepatic affections (Nandkarni, 1976).

**Rosa damascena** Mill: Common name: Rose. Local name: Kashur gulab. Has exhibited hepatoprotective activity in CCl₄ induced liver damage in rats (Chopra 1956).

**Solanum nigrum**: Common name: Black nightshade, Local name: Kambia. Given in chronic enlargement of liver (Chopra 1956).
Models Used For Inducing Hepatotoxicity

The successful therapy of the liver owes much to the identification of pathogenesis and elaboration of suitable models for hepatic injuries comparable to those encountered in clinical practice. In general, the therapeutic values of drugs are evaluated in animals experimentally made sick.

In recent years both in-vivo and in-vitro test models have been developed for the evaluation of plants/herbal drugs for their antihepatotoxic activities. These systems measure the ability of the test plant extract to prevent or cure in rats or mice liver toxicity induced by various hepatotoxins. However, a single and simple screening method is not available to identify hepatoprotective drugs with confidence.

1. In-Vivo models

a) Toxic chemical Induced liver damage

A toxic dose or repeated doses of a known hepatotoxin (Carbon tetrachloride--CCl₄--the most commonly used), paracetamol, thioacetamide, alcohol, D-galactosamine, allyl alcohol and peroxides etc.) is/ are administered to induce liver damage in experimental animals. The test substance is administered along with, prior to and or after toxin treatment. If the hepatotoxicity is prevented or reduced the test substance is effective.

Other effects of induced liver damage which can also be used in the evaluation of plant extracts are:
i) reduction in prothrombin synthesis giving extended prothrombin time.

ii) the prolonged lengthening of the time of lost reflex induced by short acting barbiturates.

iii) reduction in clearance of certain substances such as bromosulphalein.

b) Reduction in CCl$_4$ Induced Prolongation of Hexobarbitone-Induced Sleeping time

This method is used to screen anti-CCl$_4$ toxicity of drugs in animals (Burger, 1968). Hepatotoxic chemicals like CCl$_4$ reduce the levels of drug metabolizing enzymes in liver. Therefore, metabolism of hexobarbitone is reduced resulting in prolongation of hexobarbitone induced sleeping time. If a plant drug reduces this CCl$_4$ induced prolongation of 'sleeping time', the drug can be considered hepatoprotective against CCl$_4$-toxicity.

c) Anti-hepatitis Virus Activity

Simple in-vivo test systems are not available at present to determine anti-hepatitis virus activity in rodent models. However, duck and monkey models have been introduced to test antihepatitis B activity (Freiman 1988; Munshi, 1992).

d) Choleretic activity

Techniques are available to collect bile by cannulating the bile duct, in anaesthetized as well as conscious animals, to study the effect of drugs on the secretion (Shukla, 1992; Chaudhury, 1978).
e) Regeneration of hepatocytes

The effect of a drug on hepatocyte regeneration can be tested by surgical removal of a portion of the liver in experimental animals (Subramonium, 1999).

2. In-Vitro Studies

Fresh hepatocyte preparations and primary cultured hepatocytes are used to study direct antihepatotoxic activity of drugs. Hepatocytes are treated with hepatotoxin and the effect of the plant drug on the same is evaluated. The activities of the transaminases released into the medium are determined. An increase in the activities in the medium indicates liver damage. Parameters such as hepatocytes multiplication graphology, macromolecular synthesis and oxygen consumption are determined (Chrunog, 1997; Hostettman, 1987).

3. Biochemical assays

Many toxic chemicals induce liver damage by inducing lipid peroxidation and / or oxidative damage to DNA and reduction in the levels of glutathione. Antioxidant property of plant drugs is studied using liver homogenates, isolated liver cell membranes and DNA etc (Subramonium, 1999).

Carbon tetrachloride (CCL₄) is an infrequent cause of accidental and occupational poisoning. Administration of CCl₄ to rodents is a wildly used model to study both acute and chronic injury. It causes hepatocyte injury.
that is characterized by centrilobular necrosis followed by hepatic fibrosis (Chundong et al. 2002).

Paracetamol (acetaminophen)- the most widely used pharmaceutical analgesic and antipyretic throughout the world- can, when regularly taken, put a considerable amount of stress on the liver's detoxification mechanism (Draganov, P et. al, 2000) and larger amounts may cause fatal hepatic necrosis (Prescott et.al 1971). As such, it is one of the most common pharmaceutical associated with both intentional and accidental poisoning (Susan. F 2002).

In the present study, carbon tetrachloride was used as a hepatotoxin for chronic study and paracetamol was chosen for the short term one week study.
CARBON TETRACHLORIDE POISONING

The general population is exposed to carbon tetrachloride mainly through air. On the basis of reported concentrations in ambient air, foodstuffs and drinking-water, a daily carbon tetrachloride intake of around 1 microgram/kg body weight has been estimated (IPCS, 1994).

The clinical syndrome consists of hepatic failure regularly accompanied by renal failure. Indeed, CCl₄ poisoning may lead to renal failure with little or no evidence of hepatic injury. Prior to the appearance of hepatic injury there are usually neurological and gastrointestinal manifestations and a variable degree of vascular collapse (Zimmerman, 1963).

The chief histological abnormalities are in the liver and kidney but there are also changes in the lung, heart, pancrease and brain. The liver shows necrosis and some steatosis in acinar zone 3. The degree of steatosis is variable presumably reflecting, at least in part, the effects of usually associated alcoholism. Prominent ballooning may precede the necrosis. Renal abnormalities include necrosis and fatty changes of the tubules.

The lungs of the fatal cases show oedema, an alveolar pseudomembrane and thickened, fibrotic alveolar walls with epithelial proliferation. These changes may reflect renal failure rather than the pulmonary toxicity of CCl₄. The myocardial degeneration also may be a complication of renal failure or of its treatment, or may reflect a toxic effect of CCl₄ on the myocardium. Pancreatitis is a frequent finding. The mortality rate in CCl₄ poisoning is from 10-25% (Zimmerman, 1978).
In a 12-week oral study on rats (5 days/week), the no-observed-adverse-effect level (NOAEL) was 1mg/kg body weight the lowest-observed-adverse-effect level (LOAEL) reported in this study was 10mg/kg body weight, showing a slight, but significant increase in sorbital dehydrogenase (SDH) activity and mild hepatic centrilobular vacuolization. A similar NOAEL of 1.2mg/kg body weight, (5 days/week) was found in a 90-day oral study on mice, with a LOAEL of 12mg/kg body weight, where hepatotoxicity occurred.

When rats were exposed to carbon tetrachloride by inhalation for approximately 6 months, 5 days/week, 7h/day, NOAEL of 32mg/m3 was reported (IPCS, 1998). The only oral long term toxicity study available is a 2 year study in rats, which were exposed to 0, 80, or 200 mg carbon tetrachloride /kg feed.

It has been concluded that carbon tetrachloride can induce embryotoxic and embryolethal effects, but only at doses that are maternally toxic, as observed in inhalation studies in rats and mice.

Carbon tetrachloride induces hepatomas and hepatocellular carcinomas in mice and rats. The doses inducing hepatic tumours are higher than those inducing cell toxicity. It appears to be of low toxicity to bacteria, protozoa, and algae (IARC, 1979).

Administration of a single dose of CCl₄ to rats causes acute necrosis of hepatocytes in zone 3 of the hepatic lobule (Clawson 1989) resulting in complete regeneration of hepatocytes after 7 days. Recent studies have shown that in addition to necrosis, a substantial number of hepatocytes may
undergo apoptosis in response to acute CCl₄ treatment. CCl₄ hepatotoxicity depends on the dehalogenation of CCl₄ catalyzed by cytochrome P-4502E1 in the liver cell endoplasmic reticulum (Clawson 1989). Treatment with CCl₄ in-vivo activates oxygen by the P-4502E1 catalyzed reaction via an NADPH-dependent mechanism (Recknaegel 1989). Subsequent dissociation of superoxide radicals from the P-4502E1 substrate complex generates free radicals that react with microsomal membranes to induce lipid peroxidation that leads to cell membrane damage (Plaa, 1991; Kalf, 1987). The cytochrome P-4502E1 is an enzyme endowed to epithelial cells originating from the embryonic mid gut because it has been localized in the rat in pancreas and both duodenal and jejunal villous cells (Le Sage et al. 1999).

**CCL₄ -INDUCED CELL INJURY**

One of the best characterized models of lipid peroxidation is the chemical injury produced in the liver by carbon tetrachloride (CCL₄) poisoning. The halogenated hydrocarbon is used widely in the dry cleaning industry and represents a prototype for chemical injury by many similar compounds. The toxic effect of carbon tetrachloride is not due to the CCL₄ molecule but to conversion of the molecule to the highly reactive toxic free radical CCl₃ in the smooth endoplasmic reticulum (SER) by the mixed-function (P-450) oxidase systems of enzymes involved in the metabolism of lipid-soluble drugs and other compounds. It follows, therefore, that procedures that inhibit the hepatic mixed function oxidase system reduce the severity of CCL₄ injury. Thus, new born rats, which do not possess the enzyme, are resistant to the effect of carbon tetrachloride, as are protein depleted rats whose hepatic drug metabolizing system is markedly diminished. On the other hand, when hepatic mixed-function oxidase
activity is increased, as in the adaptive response to administration of Phenobarbital, the rats become hypersensitive to CCl₄ (Robbins, 1984).

At any rate, the free radicals produced locally, cause auto-oxidation of the polyenoic fatty acids present within the membrane phospholipids. There, oxidative decomposition of lipid is initiated, and organic peroxides are formed after reacting with oxygen (lipid peroxidation). This reaction is autocatalytic in that new radicals are formed from the peroxide radicals themselves. Thus rapid breakdown of structure and function of the endoplasmic reticulum is due to the decomposition of the lipid. It is no surprise, therefore, that CCl₄-induced liver cell injury is both severe and extremely rapid in onset. Within less than 30 minutes there is a decline in hepatic protein synthesis of both plasma proteins and endogeneous protein enzymes. Swelling of the cisternae of the endoplasmic reticulum can be seen early with the electron microscope, and within less than two hours there is dissociation of the ribosomes from the membranes of the endoplasmic reticulum, followed by disaggregation of the free polysomes (Smuckler, 1976).

The next event that occurs is an accumulation of lipid within the cytoplasm, beginning in the endoplasmic reticulum. This lipid accumulation is due to the inability of the cells to synthesize lipoprotein from triglycerides and "lipid acceptor protein". Triglycerides can leave the hepatic cell only after they have been incorporated into lipoprotein. Thus, failure of lipid acceptor protein synthesis leads to marked increases in intracellular triglycerides and the characteristic fatty liver of CCl₄ poisoning (Plaa. 1991)

In CCl₄ poisoning, mitochondrial injury occurs after injury to the endoplasmic reticulum, and this is followed by progressive swelling of the
cells due to increased permeability of the plasma membrane. Plasma membrane damage is thought to be caused by relatively stable fatty aldehydes, which are produced by lipid peroxidation in the SER but are able to act at different sites. Plasma membrane damage also results in a massive influx of calcium, which accumulates in the mitochondria (Kalf, 1987). The progressive damage to the cell after these events is similar to that which occurs in hypoxic injury (Morrow, 1990; 1994).

Kupffer cells also participate in the mechanism of Carbon tetrachloride toxicity, probably by releasing chemoattractants for neutrophils that produce more oxidative stress (Edwards et.al, 1993).

HEPATIC TRIACYLGLYCEROL ACCUMULATION INDUCED BY CCL₄

In both humans and animals, carbontetrachloride (CCL₄) is well known hepatotoxin (Recknagel, 1967). One of the earliest effects of CCl₄ on the liver is to cause lipid accumulation in the form of triacylglycerol (TG) resulting in fatty liver. The origin is considered to be an impairment in the secretion of TG from the liver although decreased fatty acid oxidation and increased fatty acid transport to the liver may have secondary effects.

One of the most significant and consistent effect of CCL₄ on liver lipid metabolism is the change in long chain fatty acid composition of phospholipids. In rats, the change is an increase in linoleic acid (18:2n-6) and dihomo-γ-linolenic acid (20:3n-6) and a decrease in arachidonic acid
(20:4n-6). Consistent with this change in the essential fatty acid (EFA) composition of hepatic phospholipids is the decrease in microsomal metabolism of both 18:2n-6 (δ-6 desaturation) and 20:3n-6 (δ-5 desaturation).

![Diagram of fatty acid metabolism in the liver](image)

**Fig:** Influence of Carbon Tetrachloride on the metabolism of n-6 essential fatty acids in the liver.

EFA, including γ-linolenic acid (18:3n-6) and 20:4n-6, as well as PGs have been shown to reduce or prevent fatty liver caused by CCl₄ (Cunnane & Horrobin, 1983). Furthermore, the percentage composition of 20:4n-6 in liver TG has recently been shown to be significantly inversely correlated with total liver TG. This suggests that the impaired synthesis of 20:4n-6 caused by CCl₄ may be in some way related to the increase in liver TG and

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that 20:4n-6 is part of the mechanism controlling liver secretion of TG. Thus, restoring liver 20:4n-6 levels towards normal by feeding 20:4n-6 or its precursors actually prevents TG stasis. CCl₄ is a hepatotoxin which may potentially alter TG metabolism in ways unrelated to impairment of 20:4n-6 synthesis. Nevertheless, dietary EFA deficiency is also a condition in which 20:4n-6 levels are decreased, in which fatty liver also develops, and in which 20:4n-6 supplementation reverses fatty liver. Therefore, altered metabolism of 20:4n-6 seems central to the defect in TG metabolism resulting in fatty liver. Whether PGs are involved in this effect of 20:4n-6 is not presently known but they too can prevent fatty liver caused by CCl₄ and PG synthesis inhibition with indomethacin increases CCl₄-induced liver injury (Guaner, et.al, 1982).

PATHOGENESIS OF CCL₄-INDUCED FATTY LIVER

Defective synthesis and metabolism of phospholipids, mitochondrial dysfunction, excess catecholamines and lipid peroxidation have all been considered in the etiology of CCl₄-induced fatty liver (Recknagel 1967). Although all of these contribute to some extent to fatty liver, the primary event appears to be an interruption in the normal turnover and secretion of the hepatic TG pool. The origin of the fatty acids accumulated as TG in the liver after CCl₄ has also been a matter of some debate. Both the plasma and adipose tissue have been considered as likely sources. Close examination of the hepatic parenchymal events immediately after dosing with CCl₄ has revealed that CCl₄ causes an impairment in the secretion of hepatic TG (Recknagel, 1967). Because plasma TG levels are dependent on rapid plasma fatty acid turnover through the liver (Nikkila 1969), any impairment of these processes cause rapid TG accumulation which, in the case of CCl₄, results in fatty liver. The site of the defect appears to be the endoplasmic
reticulum where newly formed hepatic TG is coupled with plasma lipoproteins for secretion. More specifically, decreased protein synthesis for incorporation into lipoproteins occurs at a sufficiently early age to be one of the primary defects induced by CCl₄ on the way to developing fatty liver (Seakins and Robbinson 1963). The apoprotein component of plasma lipoproteins is recycled through the liver and it appears that in addition to causing impaired protein synthesis de novo, CCl₄ also blocks lipoprotein formation or secretion from recycled apoprotein. However, hepatic protein synthesis can be blocked by agents such as Actinomycin D (Cunnane, 1987) without inducing fatty liver, suggesting that CCl₄-induced fatty liver is not primarily due to impaired protein synthesis per se but to altered lipoprotein metabolism.

Although the secretion of hepatic TG lipoprotein complexes is impaired by CCl₄, the uptake of fatty acids and synthesis of TG de novo by the liver is essentially normal (Recknagel, 1967). It has also been demonstrated that since plasma phospholipids fatty acids are derived from plasma TG which in turn come primarily from liver TG secretion, the origin of the fatty acids accumulated in the liver after CCl₄ poisoning was the Plasma TG fatty acid pool.

Enhancement of lipid peroxidation is an acknowledged sequel to CCl₄ administration, but its importance in the pathogenesis of fatty liver is as yet unknown. One of the carbon-chlorine bonds is cleaved in vivo yielding Cl⁻ and CCl₃⁻ radicals. These free radicals are the ones presumed to be responsible for increased malondialdehyde formation after CCl₄. Furthermore, a number of the agents which are protective against CCl₄-induced hepatotoxicity are membrane stabilizers and antioxidants, including vitamin E, selenium and zinc (Fodor G, 1965) and free radical scavengers,
e.g EDTA, desferoxamine, and propyl gallate (Slater and Sawyer, 1971). The lipoxygenase pathway inhibitor, BW 755 C, reduces liver injury caused by CCl₄, suggesting that CCl₄-induced peroxidation of 20:4n-6 by this pathway is a possibility (Guamer, 1982).

Recent research has shown that TG accumulation caused by CCl₄ does not affect the fatty acid composition of all the subclasses or pools of liver TG equally (Cunnane, 1986). Rather, comparison of the fatty acid composition of the various subclasses of TG shows that it is mainly dietary fatty acids which accumulate. Those TG subclasses which are composed to a greater extent of derived EFA e.g, 20:4n-6 and 22:6n-3, accumulate to a much lesser extent. Therefore although CCl₄ causes fatty liver largely by blocking TG secretion, the blockade appears to affect specific TG pool and not others. This suggests that the turnover rate of the various TG pools (or subclasses) varies depending on their EFA composition; those containing dietary fatty acids (16:0, 18:0, 18: ln-9, 18-2n-6) accumulate more rapidly after CCl₄, whereas those TG subclasses containing derived EFA (20:4n-6, 22:6n-3) may turnover more slowly (Cunnane 1986).
Uptake of CCl₄ by liver cells.
Conversion in SER to CCl₃⁻.
Reaction of CCl₃⁻ with microsomal polyenoic fatty acid
Generation of lipid radicals.
Reaction of lipid radicals with O₂ → Lipid peroxidation –
Autocatalytic spread along microsomal membrane.

Membrane damage
- RER
- Polysome detachment
- Lipid acceptor protein synthesis

Release of products of lipid peroxidation
- Damage to plasma membrane
- Permeability to Na⁺, H₂O, Ca++
- Cell swelling
- Massive influx of Ca++
- Inactivation of mitochondria, cell enzymes, and denaturation of proteins.

Fatty Liver

Fig : Pathogenesis of CCl₄-Induced Liver Cell necrosis.
PARACETAMOL HEPATOTOXICITY

Paracetamol, also known as N-acetyl-p-aminophenol (APAP) or acetaminophen, is one of the most widely used pharmaceutical analgesic and antipyretic agents in the world. It causes acute centrilobular hepatic necrosis in rats, mice, guinea-pigs, hamsters, rabbits, cats, dogs and pigs (Boyd and Berezcky, 1966; Davis et al., 1974; Finco et al., 1975; Gazzard et al., 1975a; Miller et al., 1976a; Mitchell et al., 1973a). However, there are marked species differences in susceptibility. Mice and hamsters are very sensitive while the rat is very resistant. The spectrum of acute paracetamol toxicity is also species dependent (Jollow et al., 1974).

At therapeutic doses it is safe but in large doses it is hepatotoxic in both man and experimental animals producing a fulminating hepatic necrosis. This necrosis is primarily centrilobular but may also extend through the mid zonal area towards the periportal areas. The necrosis is characterized by eosinophilic degeneration of the cells together with pyknosis of the nuclear material (Hinson, 1980; Boyd and Berezcky, 1966; Prescott et al., 1971; Mitchell et al., 1973a).

Sequential histological and electron microscopic studies of the liver following administration of hepatotoxic doses of paracetamol show depletion of glycogen and loss of ribosomes followed by vesiculation of endoplasmic reticulum, hydropic vacuolation, sinusoidal congestion and coagulative necrosis (Dixon, et al., 1975a; Chiu and Bhakthan, 1978). However, regeneration is rapid with no evidence of progression to cirrhosis (Dixon, et al., 1971). Liver damage is associated with variable loss of hepatic enzymes including, cytochrome P-450 (Chiu and Bhakthan, 1978;
Thorgeirsson et al. (1976) and marked increases in the serum activities of enzymes such as aminotransferases and isocitric and sorbitol dehydrogenases (Buttar, et al., 1976; Piperno, et al., 1978). The extent of histological hepatic necrosis in the rat is closely related to elevation of transaminase activities (Walker et al., 1974).

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**Fig:** Concept of metabolite-mediated drug induced liver injury.

(Pharmacological events are indicated by continuous lines and hypothetical immunological events by interrupted lines).
Liver injury will develop in all patients, who ingest sufficient paracetamol, becoming evident biochemically within 24-48 hours of the time of ingestion. Typically such massive ingestion represents deliberate suicide attempts (Hinson, 1981).

However, a small number of patients have been reported (Black, M. 1980) in whom ingestion of acetaminophen in the setting of chronic alcohol ingestion or concomitant barbiturate therapy produced a greater degree of liver damage that might otherwise have been predicted. The suggestion has been implicit that ingestion of alcohol or barbiturate “induces” the hepatic mixed function oxidase system, which serves to augment the amount of “toxic metabolite” formed for any given dose of acetaminophen.

The course of acetaminophen toxicity generally is divided into 4 phases (Susan, F 2002). Evidence of end-organ (hepatic, renal) toxicity often is delayed to 24-48 hours post ingestion.

**Phase 1 (0-24h)**
- Asymptomatic
- Anorexia
- Nausea and vomiting
- Diaphoresis
- Malaise

**Phase 2 (18-72 h)**
- Decreasing symptoms of phase 1
- Right upper quadrant abdominal pain and rising liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST]
Phase 3 (72-96 h)
Centrilobular hepatic necrosis with accompanying abdominal pain
Jaundice
Coagulopathy
Hepatic encephalopathy
Recurrence of nausea and vomiting
Renal failure
Fatality.

Phase 4 (4 d to 3 wk)
Complete resolution of symptoms
Complete resolution of organ failure (Susan, F 2002)

Mechanism of Hepatotoxicity

There is a considerable body of evidence pointing to the participation of metabolite of paracetamol rather than the parent drug in production of hepatocellular necrosis thereby establishing paracetamol hepatotoxicity as an example of the "toxic metabolite hypothesis" (Black, M 1980).

It was originally proposed that cytochrome P-450 oxidises acetaminophen to N-hydroxy acetaminophen, which then spontaneously loses water, giving the hypothetical intermediate, N-acetyl p-benzoquinone-imine (NAPQI), the presumed reactive metabolite responsible for the observed covalent binding. Recent studies however, indicate that N-hydroxy-acetaminophen is not a microsomal metabolite of acetaminophen (Hinson, 1979; Nelson, 1980). An alternative scheme to account for the formation of NAPQI, envisions the epoxidation of acetaminophen followed
by ring opening with loss of water. This mechanism has not been confirmed yet.

A third mechanism suggests that cytochrome P-450 oxidises acetaminophen to a free radical, in this case a semiquinone, and in doing so produces hydrogen peroxide (Rosen, 1973). \( \text{H}_2\text{O}_2 \) is the two electron reduction product of di-oxygen, one electron would come from the NADPH-cytochrome P-450 reductase.

The following sequence of events has been proposed:

After binding of acetaminophen to the ferric cytochrome, iron is reduced; oxygen binds and an electron is transferred from ferrous iron to oxygen, giving superoxide. Instead of a second electron being added to the system at this point, an electron is transferred from acetaminophen to the superoxide ion giving \( \text{H}_2\text{O}_2 \), a phenoxyacetaminophen free radical and regenerating the native ferric cytochrome P-450. The further transfer of an electron from the acetaminophen radical to oxygen would produce another superoxide molecule and NAPQI. There are various evidences in support of this theory. Microsomal lipid peroxidation was stimulated by acetaminophen metabolism in vitro (Rosen, 1973). More recently the enzymatic oxidation of acetaminophen analogue 3', 5'-dimethlacetaminophen (DMA) has been shown to form stable free, phenoxy free radical metabolite and benzoquinone imine (Fisher, 1984). This is interesting as DMA is of comparable toxicity to acetaminophen. It is however, incapable of covalent binding because of presence of methyl group in both the 3', 5' position of aromatic ring (Farber, 1987).
The toxicity of acetaminophen seems to depend upon a change in usual events related to metabolism of xenobiotics by mixed functions.

Paracetamol is primarily excreted as a sulphate or glucuronide conjugate. It is also metabolized by the hepatic mono-oxygenase system to a reactive metabolite. Following therapeutic doses, the reactive metabolite is efficiently detoxified by glutathione.

In acute overdose or when maximum daily dose is exceeded over a prolonged period, the normal pathways of metabolism become saturated. Excess APAP is then metabolized in the liver via the mixed function oxidase P-450 system to a toxic metabolite, N-acetyl-p-benzoquinone-imine (NAPQI). NAPQI has an extremely short half life and is rapidly conjugated with glutathione, a sulphhydryl donor, and removed from the system. Under conditions of excessive NAPQI formation or reduced glutathione stores, NAPQI is free to covalently bind to vital proteins and the lipid bilayer of hepatocytes. This results in hepatocellular death and subsequent centrilobular liver necrosis (Susan, F 2002).
Fig: Metabolism of acetaminophen, indicating probable mechanism of acetaminophen toxicity
Metabolic activation of paracetamol

Recent studies suggest that N-hydroxylation is an important metabolic step, which is catalysed by the cytochrome P-450 system. N-hydroxy-acetaminophen then undergoes a spontaneous dehydration to form the reactive metabolite, N-acetyl-imidoquinone. The metabolite then conjugates with glutathione and the resulting conjugate is subsequently excreted as a mercapturic acid derivative in urine.

The production of the toxic metabolite of paracetamol depends on the activity of microsomal enzymes and in most species hepatotoxicity is increased if these enzymes are stimulated by pretreatment with inducing agent such as phenobarbitone or 3-methylcholanthene and decreased by inhibition with piperonyl butoxide or cobaltous chloride (Jollow et.al, 1974; Mclean, 1975; Mitchell, et.al, 1973a). The marked species difference in susceptibility to the hepatotoxicity of paracetamol is related to the extent of metabolic activation as shown by the fraction created as mercapturic acid conjugate.

Thus highly sensitive species such as the mouse and hamster excrete a much higher proportion of paracetamol as the mercapturic acid conjugate, than resistant, species such as the rat. Similarly increased or decreased susceptibility caused by induction or inhibition of microsomal enzymes is associated with corresponding changes in the excretion of the mercapturic acid conjugate (Jollow, 1974).
Hepatic Glutathione Depletion and Covalent Binding

Following administration of a large hepatotoxic dose of paracetamol, the rate of formation of the toxic metabolite exceeds the maximal rate of the hepatic glutathione synthesis. Glutathione is then depleted and the excess metabolite binds covalently and irreversibly to amino acids of vital hepatic proteins and enzymes causing cell damage and death (Jollow, et.al, 1973; Mitchell, et. al, 1973b; Potter et.al, 1973; Susan F, 2002). There is a direct relationship between glutathione depletion, covalent binding of paracetamol and hepatic damage both in-vivo and in isolated rat hepatocytes (Davis, et.al, 1974; Jollow, et.al, 1973; Mitchell, et.al, 1973b; Moldeus, 1981). The hepatotoxicity of paracetamol is greatly increased, if glutathione is depleted by prior administration of diethyl maleate while precursors such as cysteine inhibit covalent binding and prevent liver damage (Mitchell, et.al, 1973b, 1974). The availability of glutathione is thus a crucial determinant of paracetamol hepatotoxicity and neither covalent binding nor hepatic necrosis occurs until levels are reduced to 20 to 30% of normal. As a result there is a well defined threshold dose to toxicity, which corresponds to the point of critical depletion of hepatic glutathione (Mitchell, et.al, 1973b). The depletion of glutathione by paracetamol is reflected by impairment of its conjugation with other substrates such as bromosulphthalein (Buttar 1976; Davis, Potter et.al, 1975a).

**Hepatotoxic dose of paracetamol**

Maximum single therapeutic dose of paracetamol does not produce liver toxicity in man and although the threshold dose has
been estimated as 10-15g (Mitchell et al., 1974) lower doses have occasionally been implicated (Fernandez and Fernando-Brito, 1977; Prescott 1978). The amount absorbed can be estimated as the product of the plasma paracetamol concentration 3 hours after ingestion and the apparent volume of distribution (10.8 l/kg). On this basis the threshold dose in man appear to be about 250 mg/kg.

There was no liver damage following absorption of less than 125 mg/kg but the incidence and severity rose steeply above 250 mg/kg. All patients who absorbed more than 350 mg/kg had severe liver damage (Prescott 1982).

**Prevention of Paracetamol Induced Liver Damage**

Paracetamol hepatotoxicity can be reduced in animals inhibiting its metabolic activation with agents such as piperonyl butoxide (Mitchell et al., 1973 a). Liver damage is always associated with glutathione depletion and this compound would seem to be the ideal antidote. Unfortunately glutathione itself does not enter cells readily and it is effective in preventing experimental paracetamol hepatotoxicity unless given in unrealistically high doses (Benedetti et al., 1975). In contrast, glutathione precursors and related compounds such as cysteine, N-acetyl-cysteine, cysteamine and methionine prevent covalent binding and hepatic necrosis in animals and have been markedly successful in preventing liver damage following paracetamol over dosage in man.
A number of sulphydryl compounds have been studied in paracetamol poisoning. Some of these are as follows:

<table>
<thead>
<tr>
<th>Cysteamine</th>
<th>Dimercaprol</th>
</tr>
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<tbody>
<tr>
<td>( \text{SH—CH}_2—\text{CH}_2—\text{NH}_2 )</td>
<td>( \text{SH—CH}_2—\text{CH—CH}_2\text{OH} )</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>Dimercaprol</td>
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<tr>
<td>( \text{CH}_3 )</td>
<td>( \text{SH} )</td>
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<tr>
<td>( \text{SH—C—CH—NH}_2 )</td>
<td>( \text{CH}_2\text{S—CH}_2—\text{CH}_2—\text{CH—NH}_2 )</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>Methionine</td>
</tr>
<tr>
<td>( \text{SH—CH}_2—\text{CH—NH}_2 )</td>
<td>( \text{SH—CH}_2—\text{CH—NCOCH}_3 )</td>
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<tr>
<td>Penicillamine</td>
<td>Methionine</td>
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<td>( \text{CH}_3 )</td>
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Cysteamine

Severe paracetamol poisoning was first treated successfully with cysteamine in 1973 (Prescott, et.al, 1974). Subsequent reports confirmed the efficiency of cysteamine, provided treatment was started within 10-12 hours (Hamlyn, et.al, 1981; Hughes et.al, 1977; Prescott, et.al, 1976). It is quite ineffective after this time.
Although effective cysteamine was not available as a pharmaceutical preparation and it produced unpleasant gastro-intestinal and central nervous system effects (Doughlas, et.al, 1976; Hughes, et.al, 1977; Prescott, et.al, 1974), safer and more convenient alternatives were, therefore, sought.

**Methionine**

Methionine given orally together with paracetamol prevents liver damage and death in rats (McLean and Day, 1975) and in some quarters there has been enthusiasm for its use in the treatment of paracetamol poisoning (Crome, et.al, 1976). However, it appears to be less effective than cysteamine or intravenous N-acetyl cysteine.

Like cysteamine, methionine must be given within 10 to 12 hours and the late treatment is ineffective. The unreliability of oral methionine may be partly due to failure of adequate absorption because of nausea and vomiting (Crome et al., 1976).

**N-Acetylcysteine**

The antidote for APAP poisoning is N-Acetyl cysteine (NAC) (Susan F, 2002). It was shown to be very effective in preventing paracetamol induced liver damage and death in animals (Piperno, Mosher and Barssenbruegge, 1976). Intravenous NAC is very effective in preventing liver damage, renal failure and death following paracetamol over-dosage and it is considered to be the treatment of choice (Prescott, 1983).

NAC is theorized to work by a number of protective mechanisms. Early after overdose, NAC prevents the formation and accumulation of
NAPQI. NAC increases the glutathione stores, combines directly with NAPQI as a glutathione substitute and enhances sulfate conjugation.

NAC also functions as an anti-inflammatory and antioxidant and has positive inotropic and vasodilating effects, thus improving microcirculatory blood flow and oxygen delivery to tissues. Vasodilating effects decrease morbidity and mortality once hepatotoxicity is well established (Susan F. 2002)

It must be given within 10 hours of ingestion and there is no evidence of benefit if treatment is delayed beyond 12 to 15 hours (Prescott, et.al, 1977; 1979). Intravenous NAC is completely effective in preventing even Trivial liver damage in most severely poisoned patients when given within 8 hours. An intravenous preparation is now marketed in some countries specifically for the treatment of paracetamol poisoning (Susan F, 2002).
Pharmacognostical characteristics of *Cichorium intybus*

**Family**: Asteraceae (Compositae)

**Vernacular names**

- **Common name**: Chicory
- **Tamil**: Kashni
- **Gujarati**: Kasani
- **Hind**: Kasni
- **Punjab**: Gul, Hand
- **Kashmir**: Hand, Kasni

**Flowering Period**: June-October

**Part Used**: Roots

**Distribution**: It is distributed in Europe, the Mediterranean region and Northern Asia, has spread to Persia, Egypt, North India, and China. In India it is found wild in Punjab, Andhra Pradesh and Kashmir. It is cultivated in Bihar, Punjab, Himachal Pradesh, Assam, Maharashtra, Gujarat, Tamil Nadu, Orissa, Andhra Pradesh and Kerela.

**Description of *Cichorium intybus* Linn.**

*Cichorium intybus* L. is an erect perennial herb 30-90 cm in height with a fleshy tap root upto 75 cm in length.
Roots dirty brownish, yellow outside, and white within; bark thin, root well developed, the central part of the root is mature and occupied with a part of the xylem including various vessels.

Leaves broadly oblong, oblanceolate or lanceolate, crowned at the base forming a rosette arranged spirally on the stem; upper leaves cordate, amplexicaul, lower leaves 7.5-15 cm long, pinnatifid.

Stems angled or grooved; branches spreading; flowers bright blue, pappus short; ligules very long, spreading, five toddled.

Fruits dry, indehiscent, 3mm long, 2 mm broad, angles crowned with a ring of white upto 5 mm long pappus which is sometimes half white and half straw coloured. Mature fruits brownish black as well as mottled; immature pale.

Seeds 2.5 mm long, ovoid, apex pointed, cotyledons plano-convex, white with average weight of 100 fruits 0.2077 grams. It flowers from June until September. Flowers are blue but can be pink or white too. All parts of the plant contain a bitter tasting milky white juice. Thus it is counted among those plants that can stimulate the appetite. The fleshy carrot like root contains the carbohydrate inulin.
Chemical Constituents

The main constituents of *Cichorium intybus* reported to be present in the root are inulin, reducing sugars and sucrose (Wight and Niekkerk, 1983). Sesquiterpene lactones such as cichoriosides A, B and C, guanine type sesquiterpene lactones such as 8-deoxy lactucin, lactucopicrin, crepidiaside B, and 11 beta,13 dihydrolactucin, two known germacrene type sesquiterpene glycoside- Picriside B and Sonchuside A- and Eudesmane type sesquiterpene glycoside Sonchuside C stand reported (Seto, et.al, 1988).

Seven compounds were reported from the roots of *Cichorium intybus* by Du, H. et.al. (1998) and four of them were identified as alpha-amyrin, taraxerone, baurenyl acetate and beta-sitosterol.
1 $R = H$
2 $R = Glc$
3 $R = H$
4 $R = CO-CH_2 C_6H_5OH$

8-deoxylactucin (1)
crepidiaside A (2)
lactucin (3)
lactucopersin (4)

5 $R_1 = H$, $R_2 = Glc$
6 $R_1 = OH$, $R_2 = H$

crepidiaside B (5)
11β 13-dihydrolactucin (6)

picriside B (7)

sonchuside A (8)
sonchuside C (9)
13 R = Glc
13a R = H
13b R = R = Ac
cichorioside C (13)

cichorioside B (12)

10 R = H
11 R = Glc
cichoriolide A (10)
cichoriosides A (11)

ixerisoside D

Cyanidin 3O β (6-O- malonyl) – D- glucopyranoside.
Traditional Ayurvedic Uses

In the traditional system of medicine, the following uses have been attributed to *Cichorium intybus*. Galenus has given it the name “friend of the liver” because of its stimulating effect on the organ. It is used as a salad vegetable (raw or strained). Bruised fresh leaves are applied externally for healing eye inflammations and boiled in broth for strengthening the digestion. An infusion of the leaves given to children reduced fever. A distilled water extract of chicory or the juice pressed out from it proved good for pregnant women and especially to soothe nursing breasts that were swollen from too much milk. It is recommended for loss of appetite and dyspepsia and also taken for rheumatic conditions and as milk laxative, mostly appropriate for children. An infusion of the leaves and flowers also aids digestion (Leung, 1996).

Pharmacological Activities

Preliminary pharmacological study of different varieties of *Cichorium intybus* showed that alcoholic extracts of all 8 varieties had a guanidine like action (marked depression of amplitude and rate) on the isolated toad heart but with variable potency, showing some promise for the use of *Cichorium* extracts to treat diseases characterized by tachycardia, arrhythmia and fibrillation (Balbaa, 1973).

Jindal et.al (1975) have shown that both the alcoholic and aqueous extracts of *Cichorium intybus* (200-800 mg/kg, i.p) exhibited anti-MES and anti-metrazole but limited anti strychnine activity. These extracts potentiated pentobarbitone and ethanol induced hypnosis in mice, exhibited
analgesia and potentiated morphine analgesia in rats. Dose related antipyretic effect against LSD induced hyperpyrexia was observed in rats. Both the extracts showed anti-inflammatory activity against formalin induced oedema.

Roy Chaudhury (1983) reported the spermatogenic inhibition in mice caused by aqueous root suspension of Cichorium intybus L. It has also been reported to possess cytotoxic activity towards cultured cancer cells (Hladon et.al, 1978; Seto, et.al, 1988).

Clinical trials conducted on alcoholic extract of Cichorium intybus against pyorrhoea in 40 dental patients revealed that when alcohol dried extracts of chicory weighing 250 mg were brushed by massaging the inflamed gums twice a day for three weeks, it significantly ($p<0.01$) reduced the gingival inflammation and bleeding (Patel, et.al, 1985).

Sultana et.al, 1995, have reported that the presence of plant extracts of Solanum nigrum and Cichorium intybus, in the reaction mixture containing calf thymus DNA and free radical generating systems, protect DNA against oxidative damage to its de-oxyribose sugar moiety- effect shown to be dependent on the concentration of the plant extract. Cichorium intybus was observed to be much pronounced as compared to the effect of S nigrum.

Inhalative occupational and ingestive immediate type allergy has been reported to be caused by chicory (Cadot, et.al, 1996).

Aqueous extract of Cichorium intybus (plant drug from Bombay market) was fractionated to yield a compound (A) identified as 8,15-
dihydroxy-2-oxo-1-(10)3, 11(13) guaiatriene12.6-olide. The extract and the compound (A) exhibited antihepatotoxic activity in-vitro testing (Gadgoli and Mishra, 1997).

Pharmacological studies of the root extracts from *Cichorium intybus* have shown their anti-inflammatory and hepatoprotective activities (Zafar and Ali, 1998; Ki, et.al, 1999).

Crude ethanolic extracts of seeds of *Cichorium intybus* and the aerial parts of *Guetterda adamanisca* etc. when administered orally on days 1-10 post coitum showed significant contraceptive activity in rats invariably associated with a significant reduction in number of implants (Keshri G and Lakshmi V, 1998).

Pretreatment of rats with Esculetin (6mg/kg), a phenolic compound found in *Cichorium intybus* and *Bougainvllra spectabillis* has been shown to protect the paracetamol induced rise in serum enzymes. It has also been reported to prevent the CCl₄- induced rise in serum enzymes, indicating thereby that esculetin possesses anti-hepatotoxic activity and the presence of this compound in *Cichorium intybus* and *Bougainvllra spectabillis* may explain the folklore use of these plants in liver damage (Gilani, Janbaz & Shah, 1998).

Methanol extract of the root of *Cichorium intybus* has been reported to possess significant anti-inflammatory activity against carrageenin induced oedema in rats hand paw at the dose of 1000mg/kg. Methanol, ethyl acetate and butanol extracts were shown to possess hepatoprotective activity. A significant reduction of blood glucose levels was observed in methanol fraction (Ki, et.al, 1999)
HD-03 a multi-herbal formulation - consisting of *Solanum nigrum* (whole plant 30%), *Cichorium intybus* (seeds, 20%), *Picrorrhiza kurroa* (roots, 20%), *Tephrosia purpurea* (whole plant, 20%) and *Andrographis paniculata* (leaves, 10%) - pretreatment at a dose of 750 mg/kg body wt. orally for 15 days in guinea pigs significantly prevented thioacetamide-induced changes in the bile flow, bile acids and bile salts excretion. HD-03 has been suggested to serve as potent choleretic and anticholestatic agent (Mitra et.al, 1999).

The leaves of *Kasini keerai* (*Cichorium intybus*) have been reported to possess considerable amounts of antioxidants. They were also found to inhibit lipid peroxidation to a significant extent, revealing their candidature for use in the preparation of anti-oxidant formulations (Saroja, S. et.al.2000)

*Kasini* (*Cichorium intybus*) extract (1 ml of 10% extract/kg body weight) when given orally for 28 days has been shown to decrease the levels of magnesium and phosphorus in urine in hyperoxaluric rats. The urine output was high in the extract treated rats (Santosh et.al. 2000).

The water soluble antioxidant properties of *Cichorium intybus* var. Silvestre were evaluated in vitro as antioxidant activity (AA) and ex vivo as protective activity (PA) and it was shown that the vegetable contained both biologic antioxidant and pro-oxidant compounds (Gazzani, 2000).

70% ethanolic extract of a group of medicinal plants, including *Selenium marianum*, *Matricaria chamomilla*, *Calendula officinalis*, *Cichorium intybus* and *Dracocephalum kotschyi*, growing in Iran have been examined by Amirghofran et.al (2000) for mitogenic activity on human peripheral blood lymphocytes and thymocytes. The results have indicated
that none of the extracts had a direct mitogenic effect on human lymphocytes or thymocytes (stimulation index, S.I< 0.07). Among the plants studied, *Cichorium intybus* and *Taraxacum officinale* have shown a complete inhibitory effect on the proliferation of lymphocytes in the presence of PHA (SI range 0.01-0.49).

Recently a molecular mechanism of anti-inflammatory action of sesquiterpene lactones, via inhibition of transcription factor NF-κβ, has been proposed (Rungeler, et.al, 1999; Han, et.al, 2001). The tumour-inhibitory effect of an ethanolic extract of chicory root was studied against Ehrlich ascites carcinoma in mice. Significant results were obtained at doses from 300 to 700mg/kg (Hazra et.al, 2002).

Kim et.al, (2002) investigated the effects of the ethanol extract of *Cichorium intybus* (CIEE) on the immunotoxicity of ethanol (EtOH) in ICR mice. The combination of CIEE and EtOH showed significant increase in the circulating leukocytes and the relative weights of liver, spleen and thymus as compared with those in mice treated with EtOH alone. The findings indicated that the immunotoxicity induced by EtOH is significantly restored or prevented by CIEE treatment.

An extract containing crude sesquiterpene lactones (CSL) from chicory was found to be effective against the motility of third stage (L3) lungworm larvae in abdominal fluid (Molan,et.al, 2003). Sakurai et.al, (2003) have reported vasorelaxant activity of caffeic acid derivatives from *Cichorium intybus* and *Equisetum arvense*. 
The methanol fraction and compound AB-IV of seeds of *Cichorium intybus* have been found to possess a potent antihepatotoxic activity comparable to the standard drug *Silybin-70* (Bahar, et.al, 2003).
Pharmacognostical Characteristics of *Taraxacum officinale*, Weber.

**Family**: Compositae

**Vernacular names**:

- English: Dandelion
- Hindi: Dulal, Barau, Kanphul.
- Punjabi: Dudli, Baran, Radam, Kanphul, Dedal.
- Deccan: Pathri
- Kashmiri: Hand
- Ladakhi: Yamaghi-khi, Rasuk.

**Flowering Period**: March-November.

**Part Used**: Roots, Leaves, Flowers and Young tops.

**Altitude**: 1500-4000m.

**Distribution**: The plant is mostly distributed throughout the temperate and arctic regions, especially in the Northern hemisphere with many varieties and micro species, found growing wild in meadows, pastures and waste ground (Grieve, 1979). The material of commerce comes from both wild and cultivated plants, mainly from Bulgaria, Hungary, Poland, Romania and United Kingdom.

About 25 species have been reported to occur in India. It is found throughout the Himalayas, in the Khasi hills of Meghalaya, Mishmi hills of Arunachal Pradesh and in the hills of South India at altitudes of 300-5400m.
It also occurs in Gujarat. It is found in pastures, meadows and on waste ground and is so plentiful that farmers everywhere find it a troublesome weed.

**Description of *Taraxacum officinale* Weber**

*Taraxacum* originated in Central Asia, but now grows almost everywhere in the world, preferring moist conditions. It has a rosette of characteristic ‘lion’s tooth’ leaves, from the centre of which arises the hollow stem bearing the yellow capitulate flower head made up of 200 or more ligulate bisexual florets. These give way to the familiar ‘fairy clock’ (Grieve, M 1931).

Rhizome is thick, vertical almost passing into a taproot, generally 15 cm long, sometimes up to 45 cm; 1.5-2.5 cm in dia., nearly cylindrical, fleshy, crowned above with several short, thickish remains of leaves, with numerous scars of rootlets, yellowish white when fresh, dark brown or blackish brown, longitudinally much wrinkled when dry; leaves radical, sessile, variable, usually glabrous; narrowly oblong, 5-20 cm. long, irregularly pinnated; lobes linear or triangular; toothed heads 0.8-5.0 cm. in dia., solitary on hollow leafless peduncles; ligulate florets golden-yellow; achenes glabrous; flattened, spindle-shaped, crowned with soft, white, copious pappus (Wealth of India, Vol 10,129).

The plant is cultivated in India for its rhizome and roots which are commonly employed in medicine with increasing demand. It is also grown for its tender edible leaves. The herb is extremely hardy and adaptable. It grows on any soil, but prefers rich sandy or light loams. Manures and
fertilizers if added at proper times hasten the growth and improve the size and succulence of the leaves. The seeds are sown in August, or somewhat later in the areas possessing mild winters; at a spacing of 25-30 cm. in the rows and 37-45 cm. in between the rows, later thinning out to 30 cm. x 45 cm. The aerial portions of the herb are harvested in the early spring, & rhizomes and roots are dug up in the autumn of the second season. The rhizomes and roots of the wild plants are dug up in the early spring before the vegetative process begins or in the autumn after it ceases. The herb has to be prevented from becoming a pest. The rhizomes and roots are washed and dried, either whole or in short pieces, 7-15 cm. long and they are stored in containers to which a few drops of carbon tetrachloride are added as a preservative (Trease, 1972; Nandkarni, 1954; Asolkar, 1992; Satayavati, 1976).

Both fresh and dried rhizomes of *Taraxacum officinale* Weber. (commonly known as Dandelion) constitute the drug, commonly known as
TARAXACUM, which is administered as a liquid extract. Fresh rhizomes and roots contain latex. According to the I.P.C, the drug should contain not more than two percent foreign organic matter and not more than four percent acid insoluble ash (Wealth of India, Raw Materials, Vol 4).

Chemical Constituents:

Root has been reported to contain sesquiterpene lactones (eudesmanolides and germacranolides); triterpenes (b-amyrin, taraxol and taraxerol); carbohydrates (inulin 2% in spring and upto 40% in autumn); carotenooids (lutein); fatty acids (myristic); flavanoids (apigenin and luteolin); minerals (potassium 1.8-4.5%); phenolic acids (caffeic acids and chlorogenic acid); phytosterols (sitosterol, stigmasterol and taraxasterol); sugars (fructose approximately 18% in spring); vitamins (vitamin A upto 14000 iu /100g); choline; mucilage (approximately 1.1%) and pectin (Bradley 1992; Budavari 1996; Newall et.al, 1996; Wichtl and Bisset, 1994; Leung AY 1996). In addition to the known compounds, a new eudesmanolide-β-D-glucopyranoside and two germacranolide acids (I & II) have been reported.

\[\text{Tetrahydrodoridentin B}\]

\[\text{I } R= \text{CH}_2\; \text{; II } R= \text{Me,H}\]
Luteolin 7-glucoside and two luteolin 7-diglucosides were isolated from dandelion flowers and leaves together with free luteolin and chrysoeriol in the flower tissue. The hydrocinnamic acid, chicoric acid, mono-caffeyl tartaric acid and chlorogenic acid were found throughout the plant and the coumarins, cichoriin and aesculin were identified in leaf extracts (Williams 1996).

Coumarins, esculin, cichoriin, caftaric acid and chicoric acid, a mixture of caftaric acid and chicoric acid dimethyl esters were isolated and identified from the leaves of *Taraxacum officinale* (Budzianowski 1997).

Five germacrane- and guaiane-type sesquiterpene lactones including taraxinic acid derivatives, were isolated from the roots of *Taraxacum officinale*, together with benzyl glucoside, dihydroconferin, syringin and dihydrosyringin. The other three lactones were identified as 11 beta, 13-dihydrolactucin, ixerin D and ainsloside (Kisiel & Barszcz 2000).

Three flavanoid glycosides: luteolin 7-glucoside and luteolin 7-diglucosides were isolated from dandelion flowers and leaves together with free luteolin and chrysoeriol in the flower tissue. The hydroxy cinnamic acids, chicoric acid, monocaffeyl tartaric acid and chlorogenic acid were found throughout the plant and the coumarins, cichoriin and aesculin were identified in the leaf extracts. Chicoric acid and the related monocaffeyl tartaric acid were found to be the major phenolic constituents in flowers, roots, leaves and involucral bracts and also in the medicinal preparations tested (Williams et. al, 1996).
Latex of dandelion roots has been reported to contain a serine proteinase-taraxalisin -that hydrolyses a chromogenic peptide substrate Glp-Ala-Ala-Leu-pNA optimally at pH 8.0. Maximal activity of the proteinase in the roots is attained in April, at the beginning of the plant development after the winter period (Rudenskaya et.al, 1998).

Dandelion contains vital nutrients and minerals as well as vitamin A, B, C and D. Rich in calcium, iron, potassium, phosphorus and sodium, dandelion is ideal for treating high blood pressure and poor digestion by stimulating the circulation of blood to the entire body. Dandelion’s anti-inflammatory properties help alleviate inflammation and muscle spasm (Grieve, M, 1983; 1985).

**Traditional Ayurvedic Uses**

*Taraxacum officinale* has a long history of being in traditional use, in many systems of medicine, in the treatment of hepatobiliary problems. The root is traditionally used to treat liver and spleen ailments. Dandelion root and leaf preparations are used as choleretic, diuretic and tonic components in a wide variety of compound dietary supplements and health food products (Leung and Foster, 1996).

In the hepatic complaints of persons, long residents in warm climates, dandelion is said to afford very marked relief. A broth of dandelion roots, sliced and stewed in boiling water with some leaves of Sorrel and the yolk of an egg, taken daily for some months, has been known to cure seemingly intractable cases of chronic liver congestion (Grieve, M 1979).
Dandelion is used as a bitter tonic in atonic dyspepsia and as a mild laxative in habitual constipation (Murray, M.T 1995). When the stomach is irritated and where active treatment would be injurious, the decoction or extract of dandelion administered three or four times a day, often proves a valuable remedy. It has a good effect in increasing the appetite and promoting digestion (Asolkar, 1992).

Dandelion, combined with other active remedies, has been used in cases of dropsy and for induration of the liver. A decoction of 2 oz of the herb or root in 1 quart of water, boiled down to a pint, is taken in doses of one wineglassful every three hours for scurvy, scrofula, eczema and all eruptions on the surface of the body (Grieve.M, 1979; Nandkarni, 1994).

It is said that juice of fresh plant is effective against liver diseases, chronic hepatitis, visceral congestion, intermittent fever and hypochondria. Roots are used to increase urine flow, as a laxative and tonic, to treat liver and spleen ailments and to stimulate appetite. Tea made by boiling flowers is used to treat heart trouble. Cooked young leaves are eaten to purify blood. Leaves are used as salad (Bhatacharya, 2001).

In Germany, dandelion root with herb is licensed as a standard medicinal tea to treat biliary disorders, digestive and gastrointestinal complaints and to stimulate diuresis. In United States, dandelion root and leaf preparations are used as choleretic, diuretic and tonic components in a wide range of compound dietary supplements and health food products (Bradley, 1992).

Its uses in North American aboriginal medicines are well documented. The Iroquois people prepared infusions and decoctions of the
root and herb to treat kidney disease, dropsy, and dermatological problems (Herrick, 1979). The Ojibwe people of Wisconsin prepared an infusion of the root to treat heartburn (Smith, 1932). The Rappahannock people of the eastern United States prepared an infusion of the root as a blood tonic and to treat dyspepsia (Speck et al., 1942). The Bella Coola people of British Columbia prepared a decoction of the roots as an analgesic and to treat stomach pain (Smith, 1929).

Pharmacological Activities

In Indian system of medicine, *Taraxacum* has been used as a mild laxative, probably it also increases flow of bile, as a diuretic, stomachic, hepatic stimulant and tonic. Rhizomes and roots are eaten raw in salads; used in soups and cooked as a vegetable. The leaves are relished in Kashmir as a vegetable (CSIR, 1986). A sort of coffee prepared from the roasted pulverized rhizomes is appetizing and given to people who cannot digest ordinary coffee. Leaves are consumed as a vegetable. They are antiscorbutic. Flower buds are pickled. Leaves and open floral heads are employed in the preparation of beer, wines etc. (Weisse, 1988).

Leaf is generally used as a diuretic to rid the body of excess fluid during detoxification for conditions as high b.p, kidney infection, obesity and swelling associated with premenstrual syndrome (Weisse, 1988)

Roots have been reported to be diuretic, tonic, aperient and used as a remedy for chronic disorders of kidney, liver, gallstones, piles and warts. Dried herb is used as a poultice around fractured limbs. It is also considered as a good binder (Chopra 1956; Kaul 1997). Decoction of leaves is taken to
relieve body aches and pains in joints after delivery. Root extracts are given orally in chronic fevers (Naqshi, et. al, 1992).


European Scientific Cooperative on Phytotherapy (ESCOP) indicates its use for restoration of hepatic and biliary function, dyspepsia and loss of appetite. Intravenous injection of fresh dandelion root decoction doubled the volume of bile secretion in dogs (ESCOP, 1997). The choleretic effect of dandelion root has been confirmed (Bradley, 1992).

Dandelion (Taraxacum officinale) root extract was examined for diuretic action. No significant increase in urine volume and sodium excretion was produced by any extract (Gee M, 1993; Hook, I et.al, 1993).

The German Standard License for dandelion decoction indicates its use for biliary disorders, gastrointestinal complaints such as a feeling of distension and flatulence, digestive complaints and to stimulate diuresis (Wichtl and Bisset, 1994).
The Commision E approved the internal use of dandelion root for disturbances in bile flow, stimulation of diuresis, loss of appetite and dyspepsia. Palestinian community uses it in congestive heart failure and premenstrual tension. British Herbal Compendium indicates its use for hepatobiliary disorders, dyspepsia, lack of appetite and rheumatic conditions as well (Bradley 1992). It is also used against gastrointestinal metaplasia and hyperplasia (Liu, 1992). Hot water extract exhibits antitumour activity (Baba, et.al, 1981: Jeong, 1991); inhibits kidney stones (Grasses, 1994), restores suppressed immune functions (Luo, 1993); possesses antiviral activity (Zheng, 1990). It is also used against chronic colitis pains along the large intestine (Chakurski, 1981).

In a study conducted on extract of *Taraxacum officinale*, evaluated on human skin melanoma cells, it was found to retain a potential antitumour activity (Oh, et.al, 1998).

Hagymasi, et.al, (2000), examined the effects of root and leaf extracts of *Taraxacum officinale* on a microsomal fraction of rat liver and found that the extracts diminished the enzymatically induced lipid peroxidation and reduced the cytochrome C with an NADPH in a concentration dependent manner.

The antihyperglycaemic effect of the antidiabetic herbal preparation *Vaccinium myrtillus* L., *Taraxacum officinale* Web., *Cichorium intybus* L., *Juniperus communis* L., *Centaurium umbellatum* Gilib., *Phaseolus vulgaris*, *Achillea millefolium* L., etc. was investigated. It was found that extract 2 (from which ethanol was evaporated) significantly decreased the levels of glucose and fructosamine in alloxan induced NOD mice (Petlevski R, 2001).
Chemical antioxidant and bioactive property studies conducted on water and ethyl acetate fractions derived from *Taraxacum officinale* flower extracts have shown that both extracts exhibited free radical scavenging activities in a stable 2,2-diphenyl-1-picrylhydrazyl radical model and reduced the breakage of super coiled DNA strand induced by non-sito specific and sito-specific hydroxyl radical (Hue & Kitt, 2003).

Besides the above uses, *dandelion root* has been indicated in eczema (combined with chickweed), skin diseases, scurvy, acne, jaundice, spleen, gall bladder, low b.p, mental fatigue, leg cramps, bowels, diabetes, asthma, aging, psoriasis, anaemia, high b.p, hypoglycaemia, rheumatism, stomach and warts, heartburn, gout and cancer (Nadkarni, 1994).

Dandelion root and leaves are believed to be quite safe, with no side effects. However, some people are allergic to dandelion which may also cause contact dermatitis (Hirono I, et, al. 1978). In some cases, it may cause obstruction of bile ducts and gall bladder (Bayerl, Jung 1996).
EVALUATION OF LIVER DISEASE

(I) Serum Enzyme Assays.

(II) Histopathology.

I ) Serum Enzyme Assays

Considering the multiplicity of liver functions it is obvious that no one test can tell us a great deal about disturbance of functions. Many tests have been proposed for the assessment of liver disease. A combination of number of tests, some that appraise hepatic function, other that reflect liver cell damage and necrosis, still others that detect infiltrative process in liver have been used effectively. These tests are also helpful in establishing the site type and extent of damage.

With the exception of those involved in coagulation, fibrinolytic and complement systems, many enzymes found in the blood stream are not primarily functional there but are in transit from one tissue to a site of degradation or removal are released into the blood in tissue disease or damage. Interest in serum enzymes for the hepatologist has resulted from the observation that increased plasma activities of some of them may be related to liver injury and to its extent. Many studies have simply correlated serum enzyme activities with disease states, and information is lacking as to the basis for the changes found in pathological conditions. Nevertheless, serum enzyme estimations have achieved wide clinical use and several are included in all lists of liver function tests and in the ‘liver profile’ or ‘battery’.

Kashmir University

Phd. Thesis
Determination of certain serum enzymes is considered useful in various types of liver injury, whether hepatocellular or cholestatic, as well as in quantifying liver damage. A combination of serum transaminases and alkaline phosphatase estimation is adequate to diagnose liver injury.
### TABLE : LIVER FUNCTION TESTS

<table>
<thead>
<tr>
<th>S No.</th>
<th>Tests</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>TESTS FOR MANUFACTURE AND EXCRETION OF BILE</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>i) Serum bilirubin</td>
<td></td>
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<td></td>
<td>ii) In faeces</td>
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<td></td>
<td>iii) In urine</td>
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<tr>
<td></td>
<td>2. <strong>Urobilinogen</strong></td>
<td>Increased in hepatocellular and haemolytic diseases, absent in biliary obstruction.</td>
</tr>
<tr>
<td></td>
<td>3. <strong>Bile acid (Bile salts)</strong></td>
<td>Increased in serum and detectable in urine in cholestasis.</td>
</tr>
<tr>
<td>1</td>
<td><strong>SERUM ENZYME ASSAYS</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. <strong>Alkaline phosphatase</strong></td>
<td>Increased in hepatobiliary disease (highest in biliary obstruction) bone diseases, pregnancy. Rise parallels alkaline phosphatase but is specific for hepatobiliary diseases.</td>
</tr>
<tr>
<td></td>
<td>2. <strong>γ-Glutamyl transpeptidase (γ-GT)</strong></td>
<td></td>
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<tr>
<td></td>
<td>3. <strong>Transaminases</strong></td>
<td>Increased in tissue injury to liver as well as to other tissues like in myocardial infarction. Increase is fairly specific for liver cell injury.</td>
</tr>
<tr>
<td></td>
<td>i) <strong>SGOT (AST)</strong></td>
<td></td>
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<tr>
<td></td>
<td>ii) <strong>SGPT (ALT)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. <strong>Other enzymes</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>i) <strong>5' nucleotidase</strong></td>
<td>Rise parallels alkaline phosphatase but more specific for diseases of hepatic origin.</td>
</tr>
<tr>
<td></td>
<td>ii) Lactic dehydrogenase</td>
<td>Increased in tumors involving the liver. Decreased in hepatocellular disease.</td>
</tr>
<tr>
<td></td>
<td>iii) Choline esterase</td>
<td></td>
</tr>
</tbody>
</table>
### III TESTS FOR METABOLIC FUNCTIONS

1 **Amino acid and protein metabolism**
   i) Serum proteins (total, A/G ratio, protein electrophoresis)
   ii) Immunoglobulins
   iii) Clotting factors
   iv) Serum ammonia
   v) Aminoaciduria

2 **Lipid and lipoprotein metabolism**
   Blood lipids (total serum cholesterol, triglycerides and lipoprotein fractions)

3 **Carbohydrate metabolism**
   Blood glucose and GIT

### IV IMMUNOLOGIC TESTS

1 **Non specific immunologic reactions**
   i) Smooth muscle antibody
   ii) Mitochondrial antibody
   iii) Antinuclear antibody and LE cell test

2 **Antibodies to specific etiologic agents**
   i) Antibodies to hepatitis B (HbsAg, Hbc, HbeAg)
   ii) Amoeba antibodies

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Hypoalbuminaemia in hepatocellular diseases
Hypoglobulinaemia in cirrhosis and chronic active hepatitis.
Non specific alterations in IgA, IgG, and IgM.
Prothrombin time and partial thromboplastin time prolonged in patients with hepatocellular disease.
Increased in acute fulminant hepatitis, cirrhosis, hepatic encephalopathy.
In fulminant hepatitis.

Increased in cholestasis, decreased in acute and chronic diffuse liver disease and in malnutrition.

Decreased in hepatic necrosis.

In hepatic necrosis.
In primary biliary cirrhosis.
In chronic active hepatitis.
In hepatitis B.
Amoebic liver abscess.

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Adapted from Systemic Pathology in: THE LIVER, BILIARY TRACT AND EXOCRINE PANCREAS.
The following biochemical tests were performed in the present study:

a & b) Serum transaminases.
c) Serum alkaline phosphatase (SAP).
d) Serum cholesterol.

Serum transaminases (Aminotransferases):

The transaminases are a group of enzymes that catalyze the transfer of an amino (\(-\text{NH}_2\)) group from an (alpha)-amino acid to an alpha-keto acid. Two such enzymes are:

- Serum Aspartate amino-transferase or AST (formerly Glutamic oxaloacetate transaminases, SGOT).
- Serum Alanine amino-transferase or ALT (Glutamic Pyruvic Transaminase, SGPT)

Serum transaminase activity increases in most disorders that produce hepatic dysfunction (Wroblewski, 1959). Moreover the tests are sensitive indicators of liver cell damage. The changing levels of serum transaminase will be due to an alteration of the semipermeable membrane of the hepatic cell allowing the passage of a large amount of enzyme into the blood.

Transaminase estimations are useful in early diagnosis of viral hepatitis. Very high levels are seen in extensive acute hepatic necrosis such as in severe viral hepatitis and acute cholestasis. Alcoholic liver disease and cirrhosis are associated with mild to moderate elevation of transaminases.
AST or SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. It is a highly specific index of hepatocellular injury. It is a much more sensitive indicator of minimal to moderate damage to the liver than are the other hepatic function tests. The transaminase in question is so called because glutamic acid and oxaloacetic acid are formed as a result of its activity.

SGOT catalyzes the transfer of amino group between aspartic acid and alpha keto-glutaric acid by following reaction.

\[
\begin{align*}
\text{L-Aspartic acid} & \quad \text{Alpha Keto-glutaric acid} \\
\text{Oxaloacetic acid} & \quad \text{Glutamic acid}
\end{align*}
\]
b) Serum Alanine Amino Transferase (ALT)/ Serum Glutamic Pyruvic Transaminase (SGPT):

ALT or SGPT is a cytosolic enzyme primarily present in the liver. SGPT catalyzes the transfer of an amino group from alanine to alpha ketoglutaric acid:

\[
\begin{align*}
\text{CH}_3 & \quad \text{COOH} \\
\text{CHNH}_2 & \quad \text{CH}_2 \\
\text{COOH} & \quad \text{CH}_2 \\
\text{C=O} & \quad \text{CH}_2 \\
\text{COOH} & \quad \text{CHNH}_2 \\
\end{align*}
\]

Alanine \quad Alpha \quad Keto-glutaric acid \quad Pyruvic acid \quad Glutamic acid

Methods for the determination of this enzyme employ aspartic acid and alpha-ketoglutaric acid as a substrate and the reaction catalyzed by transaminase results in the formation of glutamic acid and oxalo-acetic acid. In a classic method (Henry & Winkelman, 1972) introduced by Karmen the oxalo-acetic acid formed oxidizes the coenzyme diphosphopyridine nucleotide in the presence of malic dehydrogenase. The DPNH, which absorbs UV light at 340 nm, is converted to DPN, which does not have UV absorption property. Therefore, the decrease in absorbance at 340 nm resulting from oxidation of DPNH per unit time is measure of the rate of transmission. The procedure is tedious and time consuming, employs
unstable reagents and requires the use of UV spectrophotometer (Carlson, et.al, 1975).

In another method the oxaloacetate formed is converted to pyruvate by treatment with aniline citrate and pyruvate then determined photometrically after forming the coloured dinitrophenyl hydrazone and extracting into toluene (Tonbazy, et.al, 1950).

The simplest Reitman and Frankel method (1957) involves the direct combination of oxaloacetic acid with dinitrophenylhydrazine in case of SGOT and direct combination of Pyruvic acid with dinitrophenylhydrazine in case of SGPT and measurement of colour in alkaline solution (Reitman, 1957).

Physiological significance

Glutamic Oxaloacetic Transaminase (GOT) or aspartate aminotransferases (AST), is a mitochondrial enzyme present in large quantities in heart, liver, skeletal muscle and kidney and the serum level increases whenever these tissues are actually injured, presumably due to release from damaged cells.

Glutamic Pyruvic Transaminase (GPT) or Alanine Aminotransferases (ALT), is a cytosolic enzyme present in liver. The absolute amount is less than GOT but a greater proportion is present in liver compared to heart and skeletal muscles, so that serum GPT increase is more specific for liver damage than is GOT. The ratio of SGPT/ SGOT is sometimes utilized for the diagnosis of liver disease.
In Infective hepatitis SGPT is extremely high and the rise begins in the prodromal period and thus is helpful in early diagnosis and in assessing the severity of the disease. High levels are also obtained in toxic hepatitis. Little or moderate increase is observed in jaundice due to post hepatic obstruction in which bilirubin and alkaline phosphatase are appreciably increased.

Variable results are obtained in the cirrhosis of liver. In glandular fever accompanied with liver damage, rise in transaminase may be noticed. Routine screening may show unexpectedly raised transaminase levels often due to alcohol abuse, obesity or heart failure. Very high values are unusual in alcoholic liver disease. A high ratio of SGOT to SGPT (greater than two) may be useful in diagnosing alcoholic hepatitis and cirrhosis (Cohen and Kalpan, 1979).

Increases, usually less than five times the upper limit of normal, are also found in cholestasis and primary and secondary hepatic tumours. Normal values are less than 35 IU/L for SGPT.

c) Serum Alkaline Phosphatase (SAP)

The term alkaline phosphatase is applied to a group of enzymes that catalyzes the hydrolysis of a number of organic phosphate esters optimally at an alkaline pH with liberation of inorganic phosphate and the organic radical (Gutman 1959; Posen 1967). Serum isoenzymes of alkaline phosphatase, which are widely distributed in various organs of body, are useful in detecting liver or biliary tract disease. Toxic liver injury resulting in disturbance in the transport function of the hepatocyte or of the biliary
tract causes increased amount of hepatic alkaline phosphatase to enter plasma, resulting in increased serum alkaline phosphatase activity.

Several methods are available for measurement of serum alkaline phosphatase activity which depends upon the liberation and measurement of simpler methods from a phosphoric ester under controlled conditions. The oldest method is that introduced by Bodansky (1933), in which the inorganic phosphate liberated from sodium beta-glycerophosphate is measured. Another method depends upon the liberation of phenolphthalein from sodium phenolphthalein phosphate (Huggins & Tailaly, 1945) while in still another method, the quantity of beta-naphthol hydrolyzed from sodium beta-naphthyl phosphate is taken as an indirect measure of phosphatase activity (Hanheimer & Ravin, 1951). There are several modifications of a method in which phenyl phosphate or p-nitro phenyl phosphate substrate is used and the liberated phenol measured (King and Armstrong, 1934). In the present study the method of Bessey-Lowry-Brock (1946) has been used.

**Physiological significance**

The optimum pH of alkaline phosphatase is 9.7. It is found in serum, bone, kidney, liver, mammary glands, intestine, lung, spleen, leukocytes, seminiferous tubules and adrenal cortex. However, it is found chiefly in bone and liver.

Serum alkaline phosphatase increases in bone disease. Diseases, associated with this increase in the alkaline phosphatase are Paget's disease, rickets, bone atrophy, osteomalacia, osteoporosis and bone malignancy. Moderately increased values have been found in hyperparathyroidism.
Low levels of this enzyme are found in individuals hypophosphatasia (a hereditary bone disease), anaemia, infective hepatitis, or crétinism and in children, who have an unusually early movement.

The serum alkaline phosphatase activity is higher in children than in adults and gradually decreases with the age of the children than in adults and gradually decreases with the age of the children, presumably reflecting the activity of growing bone. A rise in enzyme activity is also found in pregnancy due to the increase in placental isoenzyme.

Alkaline phosphatase activity is increased in the serum of most patients with liver disease, the highest levels being observed in extrahepatic obstruction. The increase in serum alkaline phosphatase in obstructive jaundice is due to over production of the enzyme by bile canalicular membrane with leakage back into the blood.

Raised levels are sometimes observed with primary or secondary tumours, even without jaundice or involvement of bone. Increased values are also found with other spaces occupying lesions, such as amyloid, abscess, leukemia or granulomas. Non specific mild elevations are seen in a variety of conditions including Hodgkin’s disease and heart failure.

d) Cholesterol

The esterification of cholesterol is a function of the liver. Hence the determination of the free and ester cholesterol is useful in the assessment of the function of the liver, the impairment of which lowers the proportion of ester cholesterol.
Cholesterol esters constitute two thirds or more of the plasma cholesterol. Patients with severe liver disease often have a moderate or marked reduction in the esterified fraction of the cholesterol.

Despite the number of procedures available for the determination of cholesterol and its esters, there are just a few fundamental principles on which the majority of methods are based.

Cholesterol reacts with strong, concentrated acids as a typical alcohol and the products are coloured substances chiefly cholestapolyenes and cholestapolyene carbonium ions.

In virtually all procedures acetic acid and acetic anhydride are used as solvents and dehydrating agents and sulphuric acid is used as a dehydrating and oxidizing reagent. In some procedures the reaction with these agents is enhanced by the addition of various metal ions, including iron.

In the present study, total cholesterol in the serum was determined by (Kit method).

Physiological significance

Generally high plasma levels of cholesterol that reflect high levels of LDL’s may be caused by an inherited defect in lipoprotein metabolism, by disease of endocrine system, by liver disease or by renal disease. Low levels of cholesterol in the plasma may reflect an inherited deficiency of either LDL or HDL or they may reflect impairment of liver function.
Increase in the total levels of cholesterol occurs in cholestatic jaundice. Elevated serum levels occur in both intrahepatic and extrahepatic cholestasis.

An increased value of about 25% is found in pregnancy. Hypercholesterolaemia is found in nephrosis, diabetes mellitus, obstructive jaundice, myxodaema, xanthomatosis and hyperpitutarism. Very high values are found in xanthomatous biliary cirrhosis. Increases have also been reported in coronary thrombosis and angina pectoris.

Early hepatitis produces an increase in serum cholesterol, but as the disease becomes increasingly severe, the level falls, probably because of decreased synthesis by the damaged or necrosing liver cells.

Reduced values are found in hypothyroidism, pernicious and other types of anaemia, malabsorption syndrome, severe wasting and acute infections as also in haemolytic jaundice.
II) Histopathology

After preserving the livers in 10% formalin, the liver sections are processed which involves the following steps:

(a) Preparation of Tissues.
(b) Processing of Tissues.
(c) Embedding in paraffin
(d) Preparation of sections
(e) Staining.

(a) Preparation of Tissues

Fixation

Fixation is the process of killing and hardening of tissue. The first phase of fixation is the rapid killing of tissue and the second phase, the hardening of tissue. Tissue should be placed in the fixative immediately upon removal from the body to preserve the relation of the tissue elements as they were in life. Blocks are cut thin enough so that the fixing fluid penetrates the tissues in a reasonably short time. Blocks should not be more than 0.5 cm thick and are immersed in at least twenty times their volume of fixative. 10% formalin is the most widely used fixative because it is compatible with most stains. Length of fixation depends upon the size of the blocks. After fixation the tissue is washed, from 3 to 24 hours, in running water before dehydration, clearing and embedding:-
10% Formalin Solution –
37-40% formaldehyde 100 ml
Tap water 990 ml
Formalin-Saline Solution–
37-40% formaldehyde 100 ml
Sodium chloride 9.0 g
Tap water 900 ml

(b) Processing of Tissues

Dehydration, clearing and embedding:

Every specimen is marked with an identifying number or name. This identification is copied with a soft lead pencil on a string tag and is kept with the tag throughout processing.

The surface, from which sections are to be cut, is indicated by notching the opposite surface or marking it with India ink. While embedding the tissue in paraffin, the marked surface of the block is kept uppermost.

Fixed tissues are maintained in position by a firm medium so that thin uniform sections can be cut. Media suitable for this purpose are paraffin, colloidin, nitro cellulose and carbowax.

Embedding in paraffin is accomplished most rapidly and gives the best results when thin sections of soft tissues are wanted. Since paraffin is not miscible with water, the tissue must be dehydrated and then cleared in a
solution that is miscible with paraffin. Dehydration is done either by 80%, 90%, 100% alcohol or acetone. Tissues are then ready for embedding.

(c) Embedding in Paraffin

Embedding can be accelerated by the use of shallow tin pans. For embedding of multiple blocks, pans with slightly sloping sides, ranging from 1x2 inches and ¾ inch in depth are satisfactory. The pan is placed on a masonite rack, which holds it about 6 inches above the desk top. The pan is warmed gently with a Bunsen burner and filled with paraffin which has been melted and filtered. Each piece of tissue is placed in position with the appropriate string tag beside it and when all are in place the lowest part of the paraffin is hardened by rubbing an ice cube across the bottom of the pan. The pan is floated on cold water, when the paraffin has collected sufficiently so that a heavy film forms across the top.

The paraffin, when hardened throughout, contracts from the sides of the pan and the mass is lifted out and cut into blocks of appropriate size.

(d) Preparation of Sections

A properly cared knife is used, since the results produced by histologic technique depend greatly upon the knives used to cut the sections. A perfect edge for a microtome knife may be defined in simple terms as the junction of two smooth plane surfaces at an angle of about 14 degrees. Various kinds of knives are required for microtones of different types. 110 mm knife is used for cutting frozen sections, the 120 mm and the 185 mm knives for routine paraffin work.
Cutting sections

After mounting the paraffin block on the object holder excess of paraffin is cleaned and clamped in the block holder on the microtone. The knife clamp is adjusted towards the paraffin block and sectioning is begun slowly. To facilitate sectioning wet cotton is applied onto the surface of the block after cutting.

Bubbles are removed by pulling the ribbon very gently across the long edge of a glass slide half below the section in the water bath. After the section is mounted on the slide, bubbles in the tissues are removed by gentle brushing with a fine camel’s hair brush.

Resealing blocks

After cutting the sections from the paraffin block, it is resealed to prevent drying of the tissue or destruction by insects and to make subsequent cutting easier. The block sealer provides a continuous supply of molten paraffin with which to seal cut specimen blocks as they are removed from the microtone.

Forming a Ribbon

The first section is unrolled with a fine camel’s hair brush and is held down tightly against the knife. The ribbon often forms and follows number 1, 2 or 3. Camel’s hair brush is used to remove the ribbon from the knife.
**Attaching the paraffin sections to slides**

The glass slides on which tissue sections are to be mounted are marked before hand with the identifying case number usually with a glass marking pencil.

Paraffin sections can be attached to slides in several ways. A small drop of Maeyer’s egg albumin is smeared over the surface of the slide with the finger and the excess rubbed off with the help of the hand. A clean foam rubber sponge is usually preferred so that the epithelial cells from the fingers do not adhere to the slides and produce artifacts when stained.

(e) Staining

Sections picked up on albuminised slides are dried before staining or they may be stained singly by carrying each section through various solutions with a bent glass rod. Paraffin sections (5 microns thick) are stained with Haematoxylin and eosin.