INTRODUCTION

The liver is most important organ concerned with the biochemical activities in the human body. It has an important place in toxicology by virtue of its functions, both qualitatively and quantitatively. The reasons for this include its metabolic capacity and its position in the circulation. Virtually all substances absorbed from the gastrointestinal tract pass through the liver before entering the central circulation. It is involved in the maintenance of metabolic functions and detoxification, from exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism.[1,2] Human beings are exposed to toxic compounds through environment, consumption of contaminated food or due to occupational environment. In addition, human beings consume many synthetic drugs during diseased conditions, which are alien to body organs. All these compounds produce a variety of toxic manifestations.[3]

Liver disorders are of three types' necrosis, fibrosis and cirrhosis. Cirrhosis of the liver is one of the major health problems in developed countries. Cirrhosis of the liver is the fourth leading cause of death in the American adults, about 70% of which is associated with alcoholism.[4]

Clinically important hepatotoxins and their mechanism in causing hapatotoxicity[5, 6]

Mechanism of action	**Histologic lesion**	**Examples**
Direct: Physicochemical Destruction by peroxidation of hepatocytes.	Necrosis and/or Steatosis	CCl_4, Paracetamol

Indirect: Interference with hepatocellular metabolic pathways	Steatosis or necrosis	Ethionine, Ethylalcohol, tetracycline
Cholestatic: Interference with bile excretory pathways	Cholestasis duel destruction	Methylene dianiline, anabolic and contraceptive steroids
Host idiosyncrasy: Drug allergy (Hypersensitivity)	Necrosis or Cholestasis	Chlorpromazine, Phenytoin, Sulfonamides.
Metabolic: Production of hepatotoxic metabolites	Necrosis or Cholestasis	Isoniazid, Valproic acid

CCl_4 is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury. CCl_4 induces fatty liver cell necrosis and play a significant role in inducing triacyl glycerol accumulation, depletion of GSH (glutathione), increased lipid peroxidation, membrane damage, depression of protein synthesis and loss of enzyme activity. Being cytoplasmic in location the damage marker enzymes are Aspartate serum transaminase (AST), Alanine serum transaminase (ALT) and Alkaline phosphatase (ALP) are released in serum. It is now generally accepted that the hepatotoxicity of CCl_4 is the result of reductive dehalogenation, which is catalyzed by cytochrome P450 enzyme and forms the highly reactive trichloromethyl (CCl_3) free radical. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. The free radical can form covalent bond with sulphydryl group, such as glutathione (GSH), protein thiol and lipids or abstracting a hydrogen atom from an unsaturated lipid. This covalent binding of free radical to cell

macromolecules is considered the initial step in a chain of events, which eventually leads to membrane lipid peroxidation, liver damage and finally cell necrosis.

Toxicity by CCl_4 begins with the changes in endoplasmic reticulum (ER), which results in the loss of metabolic enzymes located in the intracellular structures. Liver damage is assessed by biochemical studies which includes estimation of AST, ALT and ALP, bilirubin, protein and by histopathological studies.[7, 8]

Cirrhosis is characterized by increased collagen accumulation in the liver, secondary to cell death.[9] Several mechanisms have been proposed for liver cell necrosis and collagen accumulation. One of the extensively investigated mechanisms is lipid peroxidation.[10, 11] Persistent necrosis of the liver precedes the development of cirrhosis in both humans as well as in animals[12, 13] and lysosomes play an important role in cell death and tissue damage.[14] The lysosomes release hydrolytic enzymes that cause auto-digestion of the cellular contents. Also, lysosomal enzymes play an important role in the metabolism of glycosaminoglycans[15] which are closely associated with collagen metabolism.[16, 17] Therefore, it is hypothesised that lysosomal enzymes may be altered in the liver during the course of development of cirrhosis. Alterations in the activities of the lysosomal enzymes in the liver and sera of rats in different stages of liver injury leading to cirrhosis i.e., necrosis, fibrosis by the flavonoids has not been reported.

Growth hormone (GH) is a member of the cytokine superfamily of polypeptide regulators.[18] The growth promoting effects of GH can be direct in selected target tissues, such as liver, or indirectly, via its endocrine mediator insulin growth factor-I (IGF-I). GH is the primary regulator of IGF-I synthesis and secretion in hepatocytes; in turn, IGF-I regulates GH secretion through a classical negative feedback loop.[19, 20] Growth hormone has been reported to have beneficial influence on wound healing.[21]

There are reports which suggested that administration of growth hormone prevents the lipid peroxidation in lung and liver tissue and also increased the endogenous antioxidant levels after burn injury.[22]

It has been reported that catecholamine levels contribute to the pathophysiology of paracetamol induced hepatotoxicity by compromising hepatic perfusion.[23] Catecholamines may induce oxidative damage through reactive intermediates resulting from their auto-oxidation.[24] Adrenergic inhibition mobilizes hematopoietic precursors into the circulation and has also been shown to promote liver regeneration.[25] Extensive studies in man and experimental animals suggest that the release of somatostatin and GH-releasing factor is regulated by catecholamine-containing neurons in the central nervous system (CNS).[26, 27] GH improves bioenergetics and decreases catecholamines in post infarct rat hearts.[28]

Although today the synthetic drugs are larger in their number than the natural ones still many drugs have their origin in the natural source and majority of them are derived from plants and animals for treatment of various liver disorders.[29]

Herbal medicines have had a chequered history ever since, in and out of favor with great and common man. Plants have served as the primary source of medicine and food for man; they have continued to provide mankind with new, novel therapeutic remedies to date. Since the last four decades, there has been a remarkably steady resurgence of interest in the study and use of medicinal plants. This current renewed global interest in the study and use of medicinal plants led to the characterization and identification of novel lead molecules and isolation of active chemical compounds of therapeutic importance. This revival of interest in plant derived drugs are mainly due to the wide spread belief that 'natural medicines' are safe and more dependable than the costly, synthetic drugs, many of which are toxic

and possess adverse effects. Plants are being used in the traditional system of medicine in many parts of the world, especially in rural communities, for the control, management and/or treatment of a variety of human and animal ailments. The current world wide trend towards utilization of plant-derived natural remedies has, therefore, created a dire need for accurate and up to date information on the properties, uses, efficacy, safety and quality of medicinal plant products.[30] This is an attempt to prove plant source flavonoids to treat liver cirrhosis induced by CCl_4 vapour in rodents.

Flavonoids or bioflavonoids are a ubiquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers [31]. A great number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and vasodilatory actions. The structural components common to these molecules include two benzene rings on either side of a 3-carbon ring. Multiple combinations of hydroxyl groups, sugars, oxygen, and methyl groups attached to these structures create the various classes of flavonoids: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones. Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals. The mechanism of free-radical damage includes ROS-induced peroxidation of polyunsaturated fatty acids in the cell membrane lipid bilayer, which causes a chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane lipids and proteins. Subsequently, cell contents, including DNA, are damaged.[32]

Silymarin, a flavonolignan from the seeds of 'milk thistle' (*Silybum marianum*), has been widely used from ancient times because of its excellent hepatoprotective

action. It is a mixture of mainly three flavonolignans, viz, silybin, silidianin, and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis, cirrhosis and alcoholic liver diseases. It has also been reported to be effective in certain cancers. Its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane; reduction of glutathione oxidation to enhance its level in the liver and intestine; antioxidant activity and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocytes regeneration.[33]

Quercetin is widely distributed in the plant kingdom and is the most abundant of the flavonoid molecules. Quercetin is the aglycone (meaning minus the sugar molecule) of a number of other flavonoids, including rutin, quercetin, isoquercetin, and hyperoside. These molecules have the same structure as quercetin except they have a specific sugar molecule in place of one of quercetin's hydroxyl groups on the C ring, which dramatically changes the activity of the molecule. Activity comparison studies have identified other flavonoids as often having similar effects as quercetin; but quercetin usually has the greatest activity. It is found in many often-consumed foods, including apple, onion, tea, berries, and brassica vegetables, as well as many seeds, nuts, flowers, barks, and leaves. It is also found in medicinal botanicals, including *Ginkgo biloba*, *Hypericum perforatum* (St. John's Wort), *Sambucus canadensis* (Elder) and many others. It is often a major component of the medicinal activity of the plant and has been shown in experimental studies to have numerous effects on the body. Quercetin has many beneficial effects on human health, including cardiovascular protection, anti-cancer, anti-ulcer, anti-allergy, cataract prevention, antiviral and anti-inflammatory activities.[34]

Chrysin is a naturally occurring flavone chemically extracted from the blue passion flower (*Passiflora caerulea*). Honeycomb also contains small amount of chrysin. It is also reported in *Oroxylum indicum* or Indian trumpetflower.[35] Chrysin is available as an herbal supplement, for instance body builders, are taking chrysin with the hope of raising testosterone levels or stimulating testosterone production.

Several studies in recent years reported that chrysin has multiple biological activities, such as anti-inflammatory, anticancer and antioxidant effects.

So far, the most extensively investigated mechanism of free radical induced liver injury is lipid peroxidation. Only recently, the possibility that free radicals may cause potential damage to proteins has been taken into account and wide range of changes have been reported. The formation of carbonyl derivatives is suggested to be useful measure of oxidative damage to proteins. The oxidative inactivation of enzymes by free radical and the intracellular accumulation of oxidized proteins may play a critical role in their alteration of cellular functions and cell death.[36]

Looking into ubiquitous roles of flavonoids on various pharmacological activities, the present study was designed to investigate the effect of bioflavonoid namely, silymarin, quercetin and chrysin and their combination on lysosomal enzymes (N-acetyl glucosaminidase {NAG}, β-glucuronidase {β-glc} and acid phosphatase) activity. The modulation of GH and catecholamine (adrenaline and nor-adrenaline) level were also monitored in CCl_4 vapour induced liver damage in rats, as this model produce liver cirrhosis which is similar to human alcoholic cirrhosis both histologically and systemically.