Chapter 1

Introduction

1.1 THE LIVER

The liver is the central site for metabolism, detoxification and elimination of the exogenous as well as endogenous substances. It has four lobes; namely right, left, caudate and quadrate lobe. It comprises of different types of cells, among which the major ones are: hepatocytes, kupffer cells, endothelial cells and stellate (ito) cells (Tortora and Grabowski, 2002). The main functions of the liver are: decomposing and cleansing of all the toxic substances from the body, generation of the bile-juice to help in digestion, regulation of glucose, lipid and protein metabolism, manufacturing a number of proteins, such as serum albumin and globulin, storage of fat soluble vitamins, minerals and glycogen.

Most of the xenobiotics are lipophilic in nature which facilitate them to cross the membranes of intestinal cells (Weinshilboum, 2003). The biotransformed xenobiotics get converted into hydrophilic end products which get eliminated through urine. The biotransformation process includes oxidative degeneration where cytochrome isozymes play a key role. Consequently the metabolites bind with glutathione/sulfates thus becoming more hydrophilic. The transport protein exports the formed hydrophilic product into bile or plasma which is located on the hepatocyte membrane, and is further eliminated by the kidney.

1.2 LIVER DYSFUNCTION

Major types of liver dysfunction are:

1.2.1 HEPATITIS

Hepatitis is characterized by enlargement and insufficient working of the liver. Further hepatitis can be graded into two condition acute and chronic. It can lead to liver failure and death (Tortora and Grabowski, 2002).
Fig 1.1: Detailed structure: The liver

(Adopted from: http://medcell.med.yale.edu/systems_cell/liver_and_pancreas/images/liver_cartoon.jpg)
Hepatitis is subdivided according to its morphological patterns:

- **ALCOHOLIC HEPATITIS**
  
  Excessive consumption of alcohol cause alcoholic hepatitis. Its indications are ascites (accumulation of fluid in abdominal cavity, jaundice, exhaustion and hepatic encephalopathy (dysfunction of brain because of liver failure). Hepatic ballooning, cellular necrosis and nuclear pyknosis are the major histopathogical manifestations caused by alcoholic hepatitis (Bianchi, 1983).

- **VIRAL HEPATITIS**
  
  Viral hepatitis is caused by hepatitis A, B, C, D and E type of viruses. There is a considerable impact of viral hepatitis on the public health and more than 500 million people were found to be infected. The most commonly prevalent type of viral hepatitis is caused by Hepatitis B and/or C viruses (Samer et al., 2016).

1.2.2 **CIRRHOSIS**

Cirrhosis is a disorder where scar tissue (fibrosis) replaces normal liver tissue. The normal functioning of liver gets impaired due to the condition of liver cirrhosis.

1.2.3 **JAUNDICE**

Jaundice, also known as icterus, in which the skin colour become yellow due to the imbalance in the breakdown of the bilirubin due to liver dysfunction. As a result, the bilirubin levels are increased. Jaundice is categorized majorily into three types: physiologic jaundice, pathologic jaundice and gilbert syndrome (Atkinson and Budge, 2011).

1.2.4 **HAEMOCHROMATOSIS**

Haemochromatosis, is an inborn disease caused due to extreme absorption of dietary iron, which effects in a pathological upsurge in total body iron stores which inturn affects the liver and pancreas (Kanwar and Kowdley, 2014).
1.2.5 HEPATOTOXICITY

As liver plays key role in transforming and clearing endogenous as well exogenous toxic agents from the body, therefore, it is susceptible to damage by such agents and their metabolites. Hepatotoxicity is chemically driven liver damage which progresses to mimic any form of naturally occurring liver disease and is caused by compounds known as hepatotoxins. Hepatotoxins include products of plant origin (cycasin, safrole), fungal origin, and bacterial origin (metabolite and endotoxin), minerals (Pb, Fe,), drugs (acetaminophen) and chemical agents (carbontetrachloride, bromobenzene, and alcohol). Factors that affect the potential of compound that act as hepatotoxins include; amount, point of entry, bioavailability, and distribution of the toxin, and in addition the age and health of the person. Severe cases of hepatotoxicity noticeable in the form of inflammation of the liver (hepatitis), bile retention in the liver (cholestasis), or acute liver failure. The individual mechanism which explains hepatotoxicity includes interfer with the cell’s vital metabolic pathways like mitochondrial toxicity, inhibition of the hepatic detoxification system (cytochrome P450 mono-oxygenases), interruption of vital cellular organelles, intracellular oxidative stress and generation of free radicals (Mishra and Dwivedi, 2008).

The initiation and development of the hepatic dysfunction is affected mainly by the free radicals and is not dependent on the type and cause of the dysfunction (Jaeschke, 2000; Upur, 2009). Mitochondria, hepatic drug metabolizing enzymes and the inflammatory are the key causes of free radicals (Cesaratto et al., 2004; Upur, 2009).

1.3 EXPERIMENTAL MODELS OF HEPATOTOXICITY

1.3.1 CARBON TETRACHLORIDE

Carbon-tetrachloride (CCl4) is among the key laboratory chemicals which are currently researched for hepatotoxicity studies (Brautbar and Williams, 2002). CCl4 is metabolized by hepatic microsomal enzymes (CYP2E1) to form reactive metabolites, such as trichloromethyl (CCl₃) and trichloromethyl peroxyl (CCl₃O₂) (Nada et al., 2010). These are highly unstable radicals, which bind with protein and lipids of the cell membrane, thereby triggering lipid peroxidation and causing liver damage (Debnath et al., 2013).
1.3.2 D-GALACTOSAMINE

D-Galactosamine is an amino sugar, known for inducing morphological and functional changes seen in acute hepatitis in animal models. The metabolism of D-galactosamine causes depletion of uridine triphosphate (UTP) pool, which selectively blocks RNA synthesis and transcription (Gonzalez et al., 2009; Mahima et al., 2013a).

1.3.3 ACETAMINOPHEN

Acetaminophen, a renowned analgesic and antipyretic drug (Bajt et al., 2006; Singh et al., 2011), forms NAPQI after biotransformation. NAPQI is binds to glutathione for its detoxification and elimination from the body (Dai et al., 2006). However, when an overdose occurs, the glucuronidation and sulfation routes become saturated and hepatic glutathione level gets depleted, ultimately resulting in oxidative stress. NAPQI, being a highly reactive metabolite binds covalently to vital cellular macromolecules (Sabina et al., 2013) which eventually causes cell death either via apoptosis or necrosis.

1.3.4 CADMIUM

Cadmium a redox inactive non-essential and non-corrosive heavy metal and is known for its toxic nature. It has been commonly detected in natural environmental resources. It has a high half-life and therefore higher bioaccumulation ability. Due to occupational or environmental exposure living beings are exposed to cadmium that strains antioxidant defenses causing significant deterioration of health largely due to its retention in the liver, kidney and brain for a longer time (Bagchi et al., 1996). Cadmium toxicity commences by the production of reactive oxygen and reactive nitrogen species, exhaustion of glutathione reserve, oxidative degradation of lipids and cross-linking of proteins causing damage and death of hepatocytes (Thevenod, 2003).

1.3.5 ANTI-TUBERCULOSIS DRUGS

Isonicotinic acid hydrazide (Isoniazid) and rifampicin are clinically used as anti-tubercular drugs. Isoniazid is metabolized in the liver by the microsomal enzyme, CYP2E1 to form primary metabolite N-hydroxy acetyl hydrazine. The metabolites of isoniazid bind covalently to hepatic macromolecules and cause liver injury (Saad et al., 2010).
Rifampicin is desacetyled in liver to form deacetyl rifampicin (Jamis-Dow et al., 1997) which further forms 3-formyl rifampicin (Acocella, 1978) that is responsible for hepatocellular injury (Huang et al., 2003). Simultaneous administration of rifampicin and isoniazid causes hepatotoxicity by enhanced CYP2EI-mediated bioactivation mechanism and oxidative degradation of cellular macromolecules (Martin et al., 2014).

1.3.6 THIOACETAMIDE

Thioacetamide (TAA) is routinely administered for experimental induction of liver fibrosis and cirrhosis in animal models. TAA is bio-activated by Cytochrome P450 or Flavin containing monoxygenases to form reactive metabolites. Increased formation of free radicals, oxidative stress and cell membrane disruption are believed to be major participating aspects in TAA triggered hepatic fibrosis (Low et al., 2004; Bajt et al., 2011; Thirumalai et al., 2011).

1.3.7 BROMOBENZENE

Bromobenzene (BB) a pungent smelling organic solvent which is used for large-scale crystallizations, and is a common component of motor oils. A wide range of usage has led to occupational exposures, dermal and inhalation, and the release of BB into the environment as a contaminant (Van and Schnellmann, 2003). Histopathological evidences from the literature suggest that the liver and kidney are the key organs affected on single exposure to this toxicant. Following biotransformation in the liver, BB causes hepatic and renal necrosis via its primary and secondary metabolites, respectively (Hamed et al., 2013).

1.4 THE KIDNEY

A pair of kidneys are located in the posterior section of the abdomen behind the peritoneum and are present on the either side of spine. It regulates fluid volume, maintains electrolyte content and acid-base equilibrium, endocrine and metabolic functions, including detoxification. The basic structure of kidney comprises of nephrons. Kidney of human beings is made of approximately ~1.5 million nephrons, while a rat has approximately ~35,000. The structure of a nephron begins in proximal tubule. This is followed by the loop of Henle in the middle segment. The final segment is made up of distal tubule and the ends of the nephron form the Bowman's capsule around a knot of capillaries, the glomerulus. The proximal tubule
is the first section of the nephron after the Bowman’s capsule (Fig 2). Nephron can either be juxtamedullary, which have long loops of Henle, or the cortical nephrons, which have short loops of Henle. In humans only 15% of nephrons are juxtamedullary, in rats this figure increase to 30% (Tisher, 1990; Christopher, 2000).

The kidneys are metabolically active and thus vulnerable to agents that disrupt metabolism. The structure and functions of the kidney of mammals make it a complex organ that plays a significant role in maintaining homeostasis. The kidney has a number of physiological functions such as excretion, osmoregulation, maintaining homeostasis, and regulation of pH and salts in the body. Impairment of kidney function, thus causes inhibition of these important processes and builds up a high concentration of toxic substances (Tisher, 1990; Percy et al., 2005). The renal glomeruli and interstitium are susceptible to activation of the immune mechanisms. Cellular injury to renal tissue induces changes in the allocation of membrane proteins. Cells that are severely injured thereby undergo necrosis or apoptosis, and thus sloughed-off (Pavenstadt, 2000). The sloughed cells can attach themselves to the tubules and stick together to form precipitate. This is followed by obstruction and affects the renal filtration. If the damage progresses, these irreversible changes ultimately lead to segmental glomerulosclerosis and end-stage renal failure (Christopher, 2000).

Kidney function abnormality has gained current global public health attention widely due to a significant increase in the occurrence of the disease, expensive management, and the appreciation of it as a key mediator in cardiovascular dysfunction. Environmental factors such as xenobiotics are the main cause of acute and chronic kidney dysfunctions (Ivan, 2006). Various toxicants such as environmental pollutants, industrial chemicals, radio contrast agents, chemotherapy (cisplatin), heavy metals (lithium, cadmium, lead, mercury), NSAIDS, amphotericin b, ACE’S, crystals, chemicals (bromobenzene) and calcineurin inhibitors (cyclosporine, tacrolimus) may possibly cause the progression of serious and prolonged kidney disorders (Nolin and Himmelfarb, 2010; Soderland et al., 2010; Baskaran et al., 2015).

The anatomical features and physiological functions of the kidney make it the target organ for the toxicants upon their conjugation with glutathione and might lead to
bioaccumulation of these toxicants and their metabolites, rather than serving as defense mechanisms. This initiates different mechanisms resulting in the inhibition of renal function. There may be cell death contributed by both apoptosis and necrosis in acute renal injury (Babu et al., 2003).

Fig 1.2: The structure of kidney and nephron

1.5 BROMOBENZENE: MODEL FOR HEPATO AND NEPHROTOXICITY

Bromobenzene (BB, C6H5Br), an aryl halide, is widely used in industries as an organic solvent. It is a colorless liquid with a molar mass of 157.01 g/mol and has a pungent odour. It is utilized in preparation of motor oils and food products (Table 1.1). Moreover, it is formed during the chlorination process in water purification.
Owing to its commercial use, human beings are exposed to it in industrial situations via dermal contact or inhalation and also during the contact with motor oils. It has been also detected in environmental surrounding, therefore affecting the living beings by ingestion and inhalation routes (Heijne et al., 2003; Hamed et al., 2013). It neither adsorbs to suspended solids and sediments or volatilized, nor does, it is degraded rapidly by organisms resulting in its bioaccumulation. It is known to be highly toxic and its LD 50 for human beings is 50-500 mg/kg body weight. Primary metabolites of BB such as bromo-2, 3-oxide, bromo-3, 4-oxide, generated after primary bio-transformation cause liver injury. These metabolites are followed by phase II metabolism to form BB-phenol isomers, BB-catechol, BB-quinone and BB-hydroquinone which then are localized to kidney and cause kidney injury (Reid et al., 1971; Kalantari et al., 2011; Mahima et al., 2013a).

Fig 1.3: The structure of bromobenzene

1.5.1 TOXICOKINETICS OF BROMOBENZENE

It is known that living beings are exposed to BB mainly through oral, inhalation or ingestion routes followed by absorption through the gastrointestinal tract and lungs. It was observed through experiments that blood/air partition coefficient of BB is ~200 (Aarstad et al., 1990). The in vivo studies suggested that after absorption, BB and its various metabolites were found to be distributed all the way through the various organs and prominently in the adipose tissue, kidney and liver (Mahima et al., 2013a).
In the experiments conducted in male rats treated with BB (i.p.) with a dose of 3 mmol/kg body weight of [14C] –BB, the dispersal of BB inside the body was assessed by observing its binding to protein components in various tissues (Monks et al., 1994). It was noted that the binding was highest in the liver, followed by the kidney. Although urinary elimination of the biotransformation products is the primary method of removal of BB, but biliary excretion has also been demonstrated in bile-cannulated rats.
Fig 1.4: The metabolism of bromobenzene
In the studies performed by Tanaka et al (2005; 2007) it was seen that the repeated administration of BB causes development of resistance to it. The biotransformation of BB is regulated by the members of the nuclear receptor superfamily like any other xenobiotic. This comprises of the steroid receptors, including the estrogen receptor and the androgen receptors and the non-steroid receptors, namely Pregnane X Receptor (PXR) and Peroxisome Proliferator-Activated Receptors (PPAR). They act as xenobiotic sensors that are included in the transcriptional regulation/expression of the xenobitic uptake enzymes (Xu et al., 2005). The metabolism of xenobiotics occurs in three phases of which the phase I detoxification involves the monooxygenation process, which is catalyzed by the cytochrome enzymes or CYPs. The next phase of biotransformation involves the conjugation process (Baskaran et al., 2015) where glucoronidation, acetylation, sulfation, methylation and GSH and amino acid conjugation take place. Phase III encompasses of the cellular transport of the biotransformed products across the membrane via Adenosine tri phosphate Binding Cassette receptors (Dean and Annilo, 2005).
Table 1.1: Physical and chemical properties of BB (Adopted from HSDB, 2003)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling point</td>
<td>156.0°C</td>
</tr>
<tr>
<td>Melting point</td>
<td>-30.6°C</td>
</tr>
<tr>
<td>Density</td>
<td>1.4950 g/ml at 20°C</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1.124 cp at 20°C</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>4.18 mm Hg at 25°C</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>log $K_{ow} = 2.99$</td>
</tr>
<tr>
<td>Critical temperature</td>
<td>397°C</td>
</tr>
<tr>
<td>Critical pressure</td>
<td>33, 912 mm Hg</td>
</tr>
</tbody>
</table>
CAR induces the transcriptional activation of certain CYP genes (CYP2B, CYP2C, and CYP3A), NADPH-CYP reductase, glutathione-S-transferase (GST), sulfotransferases (STs) and UDP-glucuronosyltransferase. Xenobiotic transportation is regulated transcriptionally by CAR. PXR is another key regulator of xenobiotic metabolism and is involved in transcriptional regulation of CYPs (Beigneux et al., 2002; Xu et al., 2005). Recently, these two orphan nuclear receptors are looked upon as potential targets for treating metabolic disorders as they also possess endobiotic functions that have effects on the pathogenesis of such disorders.

*In vivo* research on the experimental animals has shown that compounds with an antioxidant property provide protection against the drug-induced hepatotoxicity and nephrotoxicity (Aslam et al., 2007; Feyissa et al., 2013). In current scenario in research for discovering new agents against toxicity, BB has become one of the most experimented models for toxicity studies as it produces a pronounced measurable lipid peroxidation in various organs (Hamid et al., 2013; Hiroki et al., 2016).

1.5.2 METABOLISM OF BROMOBENZENE

Once BB enters the body, owing to its hydrophobic nature, it is biotransformed in the liver, which converts it into a hydrophilic form through various mechanisms. The initial step in the metabolism of BB is its conversion into either the BB-3, 4- or BB-2, 3- oxide driven predominantly by the CYPs. The 3, 4-oxide derivative of BB is known to be highly reactive and primarily gets conjugated with glutathione (catalysed by glutathione-S-transferase). Also, mercapturic acid has been detected as the major urinary metabolite in all the laboratory animals. It is mainly correlated with the protection against acute BB toxicity (Lau and Zannoni, 1981; Heijne et al., 2003). The BB-oxides covalently bind with haemoglobin. In further metabolism steps the BB-oxides are modified in to their analogous BB-dihydrodiols via enzyme epoxide hydrolase which subsequently converts them to bromophenols (2-, 3-, and 4- bromophenol). Also, the bromophenol metabolites finally form their corresponding BB-catechols by CYP enzymes (Jollow et al., 1974; Lind and Gandolfi, 1999).
At high doses of BB its metabolites conjugate with reduced glutathione (GSH), thereby depleting the levels of glutathione. There are alterations in cellular red/ox balance which ultimately gives rise to consequential events that cause impairment in the cell, such as lipid peroxidation, energy imbalance, and altered intracellular calcium levels. Also, mitochondria are mainly responsible for the production of reactive oxygen species through complex I and complex III (Mahima et al., 2013a). Mitochondrial functional impairment plays an important role in the apoptotic cell death through the caspases, which can be activated by ROS (Gopi and Setty, 2010a; 2010b). It has been found in experimental animals that there are transitory changes in the transcriptional expression of various genes which play pivotal role in drug metabolism (epoxide hydrolase, aldehyde dehydrogenase), oxidative stress (heme oxygenase-1, peroxiredoxin 1, metallothionein, ferritin), the acute phase response (Metallothionein, Vitronectin), cellular response to reduced glutathione levels (γ-glutamylcysteine synthetase), and intracellular signalling (Heijne et al., 2003).

Primary route of elimination of absorbed BB from the body is through urinary excretion. It is known that the secondary metabolites of BB (2-bromophenol, 2-bromoquinone, alpha and beta unsaturated epoxides of BB) and their glutathione conjugates, that are formed in its phase II metabolism are highly toxic to the kidneys (Monks et al., 1994; Bruchajzer et al., 2002) and lead to renal necrosis and tubular degeneration (Rush et al., 1984). The nephrotoxic effects of BB are due to the association of the reactive metabolites of BB and the kidney cellular macromolecules.

1.5.3 PROTEOMICS AND TRANSCRIPTOMICS OF BROMOBENZENE TOXICITY

Toxicology has been studied at molecular levels using transcriptomics and proteomics technologies. Transcriptomics technologies provide broad insight into the cellular mechanisms while the proteomics help with detailed evaluations of the cellular processes involved in toxicity. Acute toxicity (5 mmol/kg body weight) caused by BB leads to hepatic and renal necrosis as indicated by the previous studies (Hamed et al., 2013). In various studies, the effects of BB on gene expression profiles with multiple doses and time intervals were determined. Glutathione depletion and change in the body weight are the primary symptoms displayed due to BB toxicity (Jollow et al., 1974; Lau and Gillette, 1984; Lind and Gandolfi, 1999).
In a study by Heijne et al (2003; 2004), male Wistar rats were treated with BB (5.0 mmol/kg body weight, \textit{i.p.}) and were sacrificed after 24 h. Transcriptomics and proteomics were used to determine changes at the molecular level in the expression of various selected enzymes. It was observed that the levels of oxidized glutathione are not changed while the levels of reduced glutathione were depleted. Moreover, there was a strong induction in the Alpha, Mu and Pi subunits of the mRNA of GST enzyme that corresponds to the conjugation of various metabolites of BB with reduced glutathione. Gamma-glutamylcysteine synthetase and GSH synthase play important role in the synthesis of reduced glutathione synthesis. Gene expression of the enzyme γ-glutamylcysteine synthetase was found to be induced and protein expression levels of GSH synthase was increased (Heijne et al., 2003). Microsomal epoxide hydrolase plays an important role in the hydrolysis of BB epoxide intermediate, was also induced by BB (Bartosiewicz et al., 2001; Heijne et al., 2005). The BB metabolites can cause transcriptional alterations in the heme oxygenase 1 (HO-1), CYP and glutathione related genes. HO-1 catalyses the formation of bilirubin, which acts as an antioxidant and provides defence against reactive metabolites. Therefore, in conditions of oxidative stress the levels of HO-1 increase which is in consistency with the results obtained in the study done by Heijne et al (2003).

Genes responsible for the acute phase response are induced in conditions of various types of stress in order to provide protection and re-establish cellular homeostasis. The negative acute phase response proteins (albumin, ceruloplasmin, hemopexin and alpha 1 acid glycoprotein) are down-regulated and the positive acute phase response proteins (ferritins, metallothioneins and alpha-fetoprotein) are upregulated (Heijne et al., 2003; Heijne et al., 2004; Tanaka et al., 2007). There was an induction of transcriptional expression of aldehyde dehydrogenase and reductase which is important in lipid peroxidation, which can be possibly correlated with the BB-induced oxidative stress (Heijne et al., 2003). The ATPase beta subunit protein expression declined up on BB treatment, possibly due to the mitochondrial dysfunction as indicated by the previous studies (Wang et al., 1998). The mRNA level of lysosomal proteolytic enzyme cathepsin L and related subunits significantly increased indicating BB specific degradation of proteins (Heijne et al., 2003). Furthermore, significantly elevated dosages (Bartosiewicz et al., 2001) caused death of mice within 2 days (Heijne et al., 2003). More genes which were related to the stress mechanisms were found to
be induced in this study probably due to the high toxic dose of BB and species-specific differences (Heijne et al., 2003).

1.6 METABOLISM OF EXOGENOUS COMPOUNDS IN THE LIVER AND KIDNEY

The enzymes responsible mainly for the uptake of drugs and xenobiotics are grouped namely into phases I, phase II and phase III of metabolism. General functioning of phase I enzymes is regulation of various lipophilic compounds, which is accomplished by adding or unmasking of polar groups, thus rendering them more hydrophilic. Specifically, the cytochrome P450 isoenzymes and Flavin containing monooxygenases isoforms are considered the major phase I enzymes (Park et al., 1995; 2011).

CYP450 family being the largest enzyme super-families, is engaged in the biotransformation of exogenous compounds. CYP1, CYP2, CYP3 family enzymes are the primarily and predominantly associated with the drug metabolism in the humans. CYP enzymes are usually membrane bound proteins and use electrons from NAD(P)H to catalyse the initiation of molecular oxygen, resulting in the subsequent oxidation of their substrate (McDonnell and Dang, 2013).

The phase II enzymes are involved in covalently attaching a water soluble moiety to the polar group added by phase I enzymes, thus resulting in a further enhancement of polarity. Phase II drug metabolizing enzymes are generally transferases and comprise of glutathione-s-transferases, sulfotransferases, and various methyltransferases (Krishna and Sinz, 1999). Many drugs and xenobiotics passively diffuse through the cell membrane due to their high lipophilicity. Once they pass through the cell membrane and are inside the cell, they are oxidized, followed by conjugation to make them hydrophilic. Further, various transporters, mediate the efflux of metabolites which are formed during biotransformation in the cell). They are vital in metabolism, distribution and excretion of various drugs and xenobiotics (Patterson et al., 2010; Bartosiewicz et al., 2011).
1.7 IMPORTANCE OF NUCLEAR RECEPTORS IN METABOLISM OF XENOBIOTICS IN THE LIVER AND KIDNEY

The living beings have developed various mechanisms for the elimination and detoxification of toxic compounds (Gonzalez et al., 2011). Nuclear receptors (NRs) are ligand-triggered transcription factors which control the manifestation of specific genes which participate in regulating various processes for xenobiotics and endobiotics metabolism. It is identified that NRs participate in the controlling the genes related to the uptake, metabolism and elimination of extraneous compounds (Mangelsdorf and Evans 1995; Kota et al., 2005; Zollner et al., 2003).

On stimulation through any xenobiotic entering the body, the ligand binds to specified elements in the NRs followed by up-regulation or down-regulation of genes (Karpen, 2002; Zollner et al., 2003). The ligands bind to the nuclear receptors, changing their conformations and activating co-regulators and chromatin modifying mechanisms. These mechanisms include dissociation of co-repressors and recruitment of co-activators, resulting in transcriptional activation of various genes (Nishihara et al., 2004; Lee et al., 2006).

Orphan nuclear receptors are mainly involved in the regulation of drug metabolising enzymes, which take part in pivotal role in the metabolism and elimination of xenobiotics and drugs that enter human body. All NRs have similar structural domains for receptor function, including a C-terminal domain which is conserved for the binding of specific activators and dimerization interface. The ligand binding region conjugates with the DNA binding domain and induces transcriptional activation of various genes regulated by the ligand (Pellicciari et al., 2005). The N-terminal domain of the nuclear receptors is variable in nature and includes activation factor-1. Furthermore, it is responsible for the translation of specific genes (Staudinger, 2008).

The constituents of steroid family of orphan nuclear receptors, homodimerize or heterodimerize to activate the CYP2B and CYP3A promoter gene expression by various xenobiotics (Xu et al., 2005). The peroxisome proliferator activated nuclear hormone receptor (PPAR), further binds to RXR and is activated by hypolipidemic compounds directing the
activation of cytochrome gene (Xu et al., 2005; Kota et al., 2006). In cases of no ligand binding the nuclear receptor remain attached with the co-repressor, which can either be NCoR or SMRT. NCoR and SMRT encompass histone deacetylases silencing genes. Binding of ligand to nuclear receptors induces conformational alterations and causes detachment of co-repressor and recruitment of co-activators, which in turn activates the nuclear receptor (Nishihara et al, 2004; Pellicciari et al, 2005).

The three dimensional structure of various nuclear receptors have been analysed for their potential use as targets in therapies for liver-associated complications and by developing specifically designed ligands (Martin et al., 2014). Due to the extensive research, an in depth knowledge of the control of these processes has encouraged the progression of innovative drugs specially directing NRs for the protection against various diseases. Few NR agonists such as the bile acid ursodeoxycholic acid (to treat cholestasis) are already used in regular clinical practice, but further research is required to be conducted in developing novel therapeutic nuclear receptor targets as effective treatment options.

1.8 SIGNIFICANCE OF CELLULAR, BIOCHEMICAL AND MOLECULAR ALTERATIONS IN HEPATIC AND RENAL DAMAGE

1.8.1 CELLULAR ALTERATIONS

1.8.1.1 MITOCHONDRIA

Mitochondrion has pivotal role in the bio-energetics and metabolism in the eukaryotic cells. Glutathione is known to be the major defense system for mitochondrial oxidative injury prevention (Mahima et al., 2013a). Therefore, depletion in its levels is responsible for the imbalance in mitochondrial function. In cases of mitochondrial insult, pore forms in the mitochondrial outer membrane which leads to liberation of mitochondrial intermembrane contents (Bajt et al., 2006; Ramachandran et al., 2011a). Mitochondrial damage can be either caspase-dependent or caspase-independent (temperature, endonuclease G), depending on the factors responsible for it.
Mitochondria is responsible for the release of various apoptogenic factors controlled by B-cell lymphoma or Bcl-2 family. During necrosis, levels of free radicals inside the mitochondria increase. It is followed by their binding to the mitochondrial membrane constituent, pore formation, and subsiding the transmembrane potential (Ramachandran et al, 2011a; 2011b). Furthermore, the free radicals can attack other cellular macromolecules converting them in their oxidative form along with causing anti-oxidant depletion (Gopi and Setty, 2010a). Oxidative stress thus promotes mitochondrial injury (Saito et al., 2010).

1.8.1.2 LYSOSOMES

Lysosomes are sub-cellular, cytoplasmic membrane enclosed organelles, containing hydrolytic and catheptic enzymes that generate smaller biomolecules for various biological processes (Luzio et al., 2007). Lysosomes consists of acid hydrolases and are responsible for degradation of macromolecules. Cellular death via apoptosis is regulated by alterations in protein expression, protein–protein interactions, proteolytic cleavage and phosphorylation. Various factors, including Bcl-2 activated protein X (Bax), oxidative stress and DNA damage, cause lysosomal destabilization which is followed by liberation of intralysosomal hydrolases in the extracellular counterparts (Ann-Charlotte et al., 2010). Hydrolase enzyme release can cause caspase-dependent/independent cell death which can further be with/without participation of mitochondria (Fesik and Shi, 2001). The Bcl-2 family proteins (Bcl-2, Bcl-XL and Mcl-1) are downregulation targets of cathepsins (Boya and Kroemer, 2008; Ingawale et al., 2013).

1.8.1.3 OXIDATIVE STRESS

Oxidative stress is caused due to a disturbance in the levels of antioxidants and free radicals, which in turn causes oxidative modification of cellular macromolecules (DNA, proteins and lipids), cell death, and structural tissue damage. Moreover, reactive oxygen species are implicated in pathogenesis of several diseases such as cancer, diabetes, atherosclerosis and several acute and chronic liver and renal dysfunctions (Burk et al., 2004). Free radicals basically have unpaired electrons which make them highly reactive in nature. Several enzymatic and non-enzymatic antioxidants are present in the cell to combat the action of free radicals. In biological systems, antioxidant defense system is responsible for the suppression, clearance and scavenging of free radicals. Also, it helps in restoring and
reconstruction of injury and production of antioxidants (Tiwari, 2001; Naik, 2003). Superoxide dismutase (SOD) transforms superoxide anions into peroxides, catalase (CAT), transforms peroxides in O₂ and H₂O and reduced glutathione (GSH) is known to maintain membrane thiols and also acts as a substrate for other glutathione related enzymes.

1.8.1.4 LIPID PEROXIDATION

Maintenance of favorable intracellular pro-oxidant to an antioxidant ratio condition in favour of pro-oxidants are vital for the normal cellular functioning. Lipid peroxidation is closely associated with hepatotoxicity and nephrotoxicity which is due to oxidative stress, which can be associated with alteration in this ratio (Sies, 1985). The free active sites of toxicants conjugate with glutathione, thereby depleting its levels and resulting in lipid peroxidation (Sabina et al., 2013). It affects the cellular enzymes and macromolecules, including the sections of membrane bilayer and consequently disrupt the membrane resulting in cell lysis.

1.8.1.5 DISRUPTION OF CALCIUM HOMEOSTASIS

Maintenance of calcium homeostasis plays a vital role in the regulation of critical physiological functions. Oxidative stress caused due to chemically-induced toxicity may lead to the disruption of calcium homeostasis by increasing permeability of the plasma membrane, mitochondrial membrane and the membranes of smooth ER, thereby altering the levels of calcium. Impairment of calcium homeostasis leads to activation of ATPases, phospholipases, proteases and endonucleases, and disruption of mitochondrial metabolism, decreased ATP synthesis and damage of micro-filaments that support cell structure. Various toxicants known to disturb the calcium homeostasis include quinines, peroxides, acetaminophen, iron, BB and cadmium (Nicotera et al., 1990; Ingawale et al., 2013).

1.8.2 BIOCHEMICAL ALTERATIONS

Various hepato and nephrotoxins cause modifications in several liver and kidney enzymes and are normally distributed within their cells. Elevation in serum enzyme activity is routinely used as clinical biomarker for the assessment of hepatic and renal toxicity. The determination of various liver functional assessment tests; transferases, alkaline
phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GGTP), total and direct bilirubin, kidney functional parameters; urea, uric acid and creatinine in serum, and serum lipid profile; cholesterol (CH), triglycerides (TG) and lipoproteins, helps to understand the functional status of liver and kidney (Ingawale et al., 2013; Sabina et al., 2013). Moreover, it helps in the detection of liver and renal injury and determines their capacity to transport organic anions and metabolize drugs or xenobiotics (Curtis et al., 2011).

1.8.3 MOLECULAR ALTERATIONS

Cytokines are small proteins involved in cell signalling cascades and comprise interleukins, growth promoting factors, chemokines and interferons (Ramadori and Armbrust, 2001). Interleukins, including IL-10, IL1β and MCP1, are involved in drawing white blood cells to the site of inflammation. They are considered important markers of renal and hepatic injury as their levels are elevated at the site of inflammation. TNF-α is a pro-inflammatory mediator and promotes proliferation, production of inflammatory mediators and cell death. From previous studies it has been revealed that there is a proliferation in its levels in cases of liver and kidney injury (Mahima et al., 2013b). Therefore, determination of alterations in the levels of cytokines is immensely useful for the determination of early hepatic and renal damage.

1.8.4 USE OF NATURAL COMPOUNDS FOR PROTECTION IN BB-INDUCED LIVER AND KIDNEY INJURY

Present medicines that are utilized in protection against liver and kidney complications have antagonistic effects and are expensive. Thus, natural compounds having pros such as comparatively inexpensive nature, certain accessibility and ensuring fewer or no bad influences appear to be greatly fascinating. They influence the antioxidant enzymes and provide protection against free radical induced damage. Current research concentrates on the potential of various plant compounds against liver and kidney pathological conditions. Silymarin and N-acetyl-L-cysteine are being already used in the clinical treatment of liver and kidney injury and they reveal a potent protective activity, but with certain restrictions such as gastric irritation, allergies and limited efficacy (Gopi and Setty, 2010a; Mahima et al., 2013b). This indicates that there is still the need of finding more successful and consistent agents with insignificant side effects for the prevention of acute liver and renal failure. Few
herbal therapeutics have been proved to be effective against BB-induced liver and renal injury.

1.8.4.1 AGED GARLIC EXTRACT

In a previous study, precision cut liver slices from phenobarbitol induced rats were treated with BB (1 mM) and acute injury was indicated by increase of liver functional marker levels and decreased glutathione content. Pre-treatment of rats with aged garlic extract (AGE, Kyolic®) (2 and 10 ml/kg each day for a week) decreased the toxicity probably by restoring the levels of glutathione (Wang et al., 1998).

1.8.4.2 ROSA RUGOSA AND ROSAMULTIN

In an experiment conducted by Park et al (2004), male Sprague-Dawley rats were orally provided with methanolic extract of Rosa rugosa and its principle component rosamultin. BB (460 mg/kg body weight, i.p.) was provided to the rats in the end for four times at 12 h intervals. The activity of the CYPs, demethylases and hydroxylases in the hepatic system increased considerably in the BB induced group of studied animals. Pretreatment of BB treated rats with R. rugosa and rosamultin altered the activities of these enzymes. It was concluded that the hepatoprotection by the R. rugosa and rosamultin, in BB-induced toxicity can be due to the enhanced activity of the hepatic hydrolases. Epoxide hydrolase immediately transforms the toxic biotransformation products of BB formed by the phase I metabolism which involves CYPs.

1.8.4.3 ALISMA ORIENTALE

In a similar study, male Sprague-Dawley rats, pre-administered with methanolic extract of Alisma orientale (250 and 500 mg/kg body weight) for one week, were injected with BB (460 mg/kg body weight, i.p.) on the last two days for four times at 12 h intervals. It was observed that the methanolic extract of A. orientale rhizome enhanced the hepatic antioxidant enzyme activity in BB treated animals (Hur et al., 2007).
1.8.4.4 GINGER EXTRACT

Male albino rats were pre-administered with 100-300 mg/kg body weight of ethanolic ginger extract for three weeks and were provided 460 mg/kg body weight of BB by intragastrically in the last week. BB at 460 mg/kg body weight for a week elicited alterations of certain oxidant and drug metabolizing processes in the hepatic tissue. Also, the expression of apoptotic markers increased and DNA fragmentation was seen in BB treated group of rats. It was concluded from the results that ginger extract exhibits significant protection in BB-triggered hepatic oxidative injury by bringing the antioxidants and drug metabolizing enzymes to normal levels (El-sharaky et al., 2009).

1.8.4.5 CASSIA FISTULA

In a study conducted by Kalantari et al (2011) Cassia fistula fruit extract (200, 400, 600 and 800 mg/kg body weight) was given to mice for about one week followed by BB (460 mg/kg body weight) treatment. It was observed that the fruit extract provided significant protection against BB-induced nephrotoxicity which was probably due to the antioxidant enhancing activity of its components.

1.8.4.6 PHYLLANTHUS FRATERNUS

In another study, male rats were given aqueous extract of Phyllanthus fraternus (100 mg/kg body weight, oral) for almost a week and then BB (10 mmol/kg body weight, intragastric intubation) was given. Rats were sacrificed 19 h after the last dose and change in the mitochondrial antioxidants was assessed in the studied rats. There was a decrease in mitochondrial antioxidant status and increase in lipid peroxidation indicating mitochondrial dysfunction. The results obtained in this study showed that the mitochondrial impairment induced by BB was prevented in a far better manner as compared with the aqueous extract of Phyllanthus fraternus than vitamin E (Gopi and Setty, 2010b).

1.8.4.7 ZANTHOXYLUM PIPERITUM AND PROTOCATECHUIC ACID

In a previous study, the protective effect of Zanthoxylum piperitum (ZP) and its active component protocatechuic acid, on the activity of hepatic antioxidants and functional markers was evaluated in BB treated rats. Male rats were given methanolic extract of the ZP
leaves (250 and 500 mg/kg body weight, oral) or PA (5, 10 and 20 mg/kg body weight, oral) for seven days followed by BB (460 mg/kg body weight, i.p.). The activities of the enzymes involved in hepatic metabolism, such as, demethylases and hydroxylases, and hepatic antioxidants was found to be altered in BB treated group of animals. *Zanthoxylum piperitum* (ZP) and its active component protocatechuic acid decreased oxidative stress and restored the activity of aniline and epoxide hydroxylase in rats intoxicated with BB (Hur et al., 2003).

1.8.4.8 BLACK SEED OIL

Male Wistar albino rats were orally administered with black seed oil 30 min. prior to BB continuously for three weeks. Antioxidant status was estimated in the liver of the studied group of rats (Hamed et al., 2013). Moreover, liver and kidney functional parameters were assessed and histopathological analysis was also done. It was concluded from the results, black seed oil improved the hepato-renal defense mechanism and decreased progression of disease related complications.

1.8.4.9 HISPIDULIN

The beneficial properties of hispidulin, a flavone, in BB-triggered toxicity in animals was examined in a previous study. Liver damage was induced by the BB administration as evidenced by increased lipid peroxidation along with intensive liver glutathione depletion in mice. Hispidulin (500 mg/kg body weight, i.p.) inhibited liver injury by diminishing glutathione depletion and oxidative injury. The ameliorative effects of hispidulin in liver toxicity were in correlation to the antioxidant properties of hispidulin (Ferrándiz et al., 1994).

1.8.4.10 *HEMIDESMUS INDICUS*

In a study conducted by Gopi and Setty (2010b), male rats were pre-administered with aqueous extract of *Hemidesmus indicus* (100 mg/kg body weight). On the 8th day rats were given BB (10 mmol/kd body weight, intragastric intubation) and were sacrificed 19 h after the last dose. BB administration induced liver injury as revealed by augmented lipid peroxidation and decreased mitochondrial enzyme activities. This study established that the pre-administration of *Hemidesmus indicus* provides significant protection in the BB-induced
mitochondrial oxidative stress, which can be accredited essentially to its antioxidant potential.

1.9 WITHANIA SOMNIFERA

Withania somnifera (Fig 1.5) popularly known as Ashwagandha, Dunal (Solanaceae) is a valued Indian medicinal plant known to possess pharmacological properties such as antistress, antioxidant, immune-modulating and anti-arthritic activities (Dhuley, 1998; Rasool and Varalakshmi, 2006). These properties may be due to the presence of various biologically active chemical constituents such as alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitonidoside XI and X) (Singh et al., 2003; Palliyaguru et al., 2016). The roots of W. somnifera (WS) is the most pharmacologically active part of the plant and are known to possess free radical scavenging and antioxidant activity (Rasool and Varalakshmi, 2006).
Fig 1.5: The roots of *Withania somnifera*
1.9.1 PHARMACOLOGICAL PROPERTIES:

1.9.1.1 HEPATOPROTECTIVE AND NEPHROPROTECTIVE EFFECTS

It is already known that *W. somnifera* has restorative effects in carbendazim-triggered hepatic and renal histoarchitectural alterations (Akbarsha et al., 2000) and protective effects against gentamicin induced kidney injury (Shimmi et al., 2011) in rats. In a study conducted by Mansour and Hafez (2012), alcoholic extract of *W. somnifera* (100 mg/kg body weight) was established to prevent γ-irradiation induced hepatotoxicity as validated by the reformation of the liver functional assessments and antioxidant status, reduced DNA fragmentation and significant decrease in Heme oxygenase-1 induction. It was also found to exhibit hepatoprotective effects against paracetamol-induced toxicity (Sabina et al., 2013).

1.9.1.2 ANTI-INFLAMMATORY AND ANTI-ARTHRITIC EFFECTS

*W. somnifera* exhibits anti-inflammatory and anti-arthritic effects by suppressing the inflammation (Rasool and Varalakshmi, 2006). Furthermore, *W. somnifera* possesses antipyretic properties and its anti-inflammatory and the anti-arthritic activity was found to be higher than that of the standard drug indomethacin. Rats administered with *W. somnifera* (orally) one hour prior to the inflammatory agent showed that its anti-inflammatory action was equivalent to the hydrocortisone sodium succinate, which is commonly prescribed anti-inflammatory drug (Begum and Sadique, 1988).

1.9.1.3 NEUROPROTECTIVE EFFECTS

A renowned natural drug, BR-16A (Mentat®), which contains *W. somnifera* as one of the key constituent demonstrated prevention in experimental catalepsy in animals. BR-16A along with *W. somnifera* diminished catalepsy in experimental animals. In another study, the antiparkinson effects of *W. somnifera* extract was estimated using experimental Parkinson's effect in animals (Ahmad et al., 2005). *W. somnifera* has intense CNS anti-depressant actions and has anti-convulsant activities in severe as well as long-lasting epilepsy (Kulkarni et al., 1993; Ziauddin et al., 1996).
1.9.1.4 ANTI-OXIDANT EFFECTS

Antioxidant status of animals treated with *W. somnifera* was examined in hypercholesteremic animals and decrease in lipid-peroxidation was observed in *W. somnifera* treated rats. Furthermore, in stress-induced animals the *W. somnifera* decreased the levels of lipid peroxidation (Dhuley, 1998). Furthermore, few withanolide glycosides isolated from leaves of *W. somnifera* were assessed to evaluate their capacity to prevent cyclooxygenases (COX). It was shown that withanolides showed selective COX inhibition and prevented lipid oxidation (Jayaprakasam and Muraleedharan, 2003).

1.9.1.5 ANTI-CANCER EFFECTS

*W. somnifera* reduces the expression of nuclear factor kappa B, intercellular tumor necrosis factor, and increases the apoptotic signalling in cancerous cell lines. Pre-administration of *W. somnifera* for seven months decreased the changes in histoarchitecture of the lungs of animals. *W. somnifera* also inhibited growth of different cancer cell lines by reducing their viability and therefore embraces potential as an anti-cancer agent (Jayaprakasan et al., 2003). Withaferin A, which is the active component of *W. somnifera*, suppressed the breast cancer cell viability (Silvia et al., 2008). According to previous studies it has been known that the roots of *W. somnifera* contain several alkaloids, withanolides, a few flavonoids and reducing sugars and are also rich in iron (Rasool and Varalakshmi, 2006).

1.9.2 TOXICITY PROFILE OF WITHANIA SOMNIFERA

*W. somnifera* is known to be a safe Ayurvedic medicinal plant. A previous study utilized ashwagandholine (*W. somnifera* roots) which is a mixture of gycol and gum acacia to establish critical toxicity. The critical LD 50 was established 465~ mg/kg (Malhotra et al., 1965). The dosage of up to 250 mg/100 g were found to be safe for and did not affect the overall nervous system. While, the motor activity response was altered at greater doses. In an alternative extended research, boiled *W. somnifera* roots were given to the rats in dailyin water for a period of 8 months. The body weight, litter size and toxicity, was observed (Sharma et al., 1986) at the dosage of 100 mg/kg body weight/day. Histopathological analysis of the various organs including the liver and, kidneys, depicted normal histoarchitecture. The *W. somnifera* treated group of rast gained weight andhealthier offsprings were produced by the rats receiving *W. somnifera* (Sharma et al., 1986).
In subacute toxicity study, with a combination of *W. somnifera* and Ginseng conducted by Aphale et al (1998) for 90 days various selected parameters including food consumption, body weight, haematological parameters were observed. The results have shown that the plant formulations did not cause toxicity in any form (Aphale et al., 1998). The histopathological analysis of various organs of the animals was in concordance with the results. However, in one study with whole plant extract in nutritional regimen, liver abrasions were noted along with vascular and tubular obstructions of the kidneys in the mice. The leaf extract of *W. somnifera* was conveyed to retain antigenotoxic possibilities (Russo et al, 2001; Rani et al., 2005). Many other studies conducted on the safety assessment of the *W. somnifera* which thereby reveal that this plant is non-hazardous in a widespread variety of realistic amounts and it can be presumed that the amounts in which its compounds are specified in humans are probably safe.

1.9.3 CHEMICAL CONSTITUENTS OF WITHANIA SOMNIFERA

Plants produce a variety of phytocompounds, such as alkaloids, terpenoids and propanoids in response to external and internal stimuli. Various alkaloids, withanolides and sitoindosides have been separated and identified in the roots, stems and leaves of *W. somnifera* (Singh et al., 2001). Withanolides (WT) are constituted of a steroid support bound to a lactone or its byproducts. A considerable and comprehensive research has been undergone in the past to evaluate the pharmacological properties of the WT, particularly Withaferin A, WT E and WT D. Notable activities reported for these compounds include anti-inflammatory, anti-tumor, immunosuppressive and antioxidant properties (Kaileh et al., 2007). *W. somnifera* extracts have been broadly considered for their pharmacological activities. To illustrate the bioactive components in Ashwaganda, several investigations have been done in the chemical constituents of *W. somnifera* by diverse methodologies. Steroidal lactones such as WT, flavonoids, alkaloids and tannins are the chief biochemical components *W. somnifera* (Sabina et al., 2013).
1.10 WITHAFERIN A

Withaferin A is the highly oxygenated and one of the most therapeutically active chemical constituent of the roots and leaves of the plant *W. somnifera*. Withaferin A (Fig 1.6) is categorized into steroidal class of secondary metabolites.

Fig 1.6: The chemical structure of Withaferin A
Oxygenated C28-steroidal derivatives form the WTs (Fig 1.6). The cycloalkane rings are joined together in a WT skeleton and the C-22 and C-26 get attached to oxygen to produce a hexagonal lactone form (Berghe et al., 2012). WA retains a varied range of properties, including inflammation inhibiting, tumor preventive, radiosensitizing and antiangiogenic effects (Dhuley, 1998; Jayaprakasan and Muralleedharan, 2003; Okamoto et al., 2016). Furthermore, it is also demonstrated to possess hepatoprotective activity against paracetamol-induced toxicity (Ravirajsinh et al., 2015). Although the biochemical method by which WA achieves these effects is still principally unidentified, numerous descriptions have been anticipated, encompassing covalent attachment and modification of the enzymic active radicals (Okamoto et al., 2016).

1.10.1 MOLECULAR TARGETS AND THERAPEUTIC IMPLICATIONS OF WITHAFERIN A

Due to its interesting structure and various properties, Withaferin A has gained serious attention from researchers. NF-κB mediates pathways included in the induction of proteins that are responsible for cellular development, angiogenesis and death. WA is known to prevent TNF mediated stimulation of NF-κB pathway via hindering instigation of IκB kinase. It blocks the NF-κB controlled gene expression, such as COX-2 to exert anticancer activity as suggested by Bernier et al (2006). Activation of the nuclear factor is strongly controlled and it is present in inactive form in the cytoplasm conjugated with its inhibitor IκB. Several pro-inflammatory intermediates activate specific cognate receptors and activate NF-κB via inducing the formation of IκB-kinase complex (IKK). It comprises of the IKKγ (NEMO) and IKKα and IKKβ, and has similar sequences (Liang et al., 2006).

Phosphorylation of inhibitory IκBα protein allows transportation of NF-κB inside the nucleus leading to transcriptional induction of numerous pathways. WA has strong intermolecular interactions with IKKβ which acts as the monitoring subunit of the IKK-kinase complex. WA interacts with the N-terminal helix of IKKγ causing obstruction for IKKβ to conjugate to the complex. This incapability of IKK-complex formation results in blockage of release of NF-kB to the nucleus. Also, it has been hypothesized that WA inhibits the TNF-induced NF-κB activation realted to IKKβ by probably suppressing IKKβ kinase activity via disturbing IKK a/b complex formation (Bernier et al., 2006; Gupta et al., 2010).
Substantiation of both the above propositions showing NF-kB hindering effects of WA needs additional in depth exploration. The NF-kB hindering capability of WA is under examination in various cellular environments via numerous incitements. NF-kB controlled gene expression is being studied in various cell types as it is plays important role in pathogenesis and progression of various diseases, including leukemia, multiple myeloma (MM), rheumatoid arthritis, obesity, type 2 diabetes and atherosclerosis (Berghe et al., 2012).

Withaferin A is known to affect protein kinase C as established in a study by Sen et al (2007) Protein kinase C (PKC) is related to cellular development, hormonal regulation secretion and apoptosis. PKC has more than 11 diverse classes of isoforms but WA is known to inhibit the activity of PKC purified from rat brain. The kinase modifying activities of the WA is studied extensively. Since the effects of WA varied considerably, which might redirect the cellular type, dose/time dependent effects. Therefore, further investigation is required to know the possibility that these effects are directly or indirectly targeted by WA.

Besides NF-κB various other transcription factors involved in inflammation, cell signalling and apoptosis, including liver X receptor alpha (LXR-α) are demonstrated to be affected by WA (Lee et al., 2010; Munagala et al., 2011). Since, gene transcription, a highly regulated process, targeting one transcription factor may affect the activities of all the proteins involved in the cascade. LXR-α is a part of NR family which via in silico molecular analysis revealed possible interactions of WA with its ligand binding domain. LXR is responsible for targeting the regulatory mechanisms included in hepatic cholesterol fatty acid and glucose metabolism. LXR is anticipated to be of favorable medicinal value for the treatment of cancer due to its involvement in anti-proliferative and apoptosis regulating cascades (Blaschke et al., 2004). However, the ligand attaching NR domains of LXR-α being highly conservative for WA, the LXR-α and WA for involves additional assessment.

1.11 RESEARCH RATIONALE AND HYPOTHESIS

Bromobenzene (BB) is widely used in the industries for the manufacture of various drugs and chemicals. It is known through previous studies that in the current scenario in research for discovering new agents against toxicity BB has become one of the most
researched up on compound for the toxicity studies. The measurable malondialdehyde (MDA) concentration in various organs is greater in BB toxicity as compared to the other xenobitoic compounds (William, 2003; Gopi and Setty, 2010a; 2010b; Hamed et al., 2013). BB is metabolized in the liver primarily by CYP enzymes to form intermediates which thereby act as reactive free radicals and are initially detoxified by conjugation with glutathione. This causes reduction in the levels of glutathione and ultimately the primary metabolites of BB bind to cellular macromolecules and cause hepatic necrosis (Waters et al., 2006). Similarly the secondary metabolites of BB contribute to renal oxidative stress and cause nephrotoxicity. The reactive metabolites of BB themselves form free radicals. Most importantly, due to reductions in levels of cytosolic and mitochondrial glutathione there is an increment in levels of reactive oxygen species inside the mitochondria (Lind and Gandolfi, 1998; Heijne et al., 2004; Kalantari et al., 2011). There is a possibility of increasing the protective approach by augmenting the mitochondrial antioxidant status via utilizing free radical scavenging property possessing natural compounds. The effectiveness of this approach has been proven by several studies. Recent studies have focussed on the potential of various natural compounds against liver and kidney pathological conditions. Silymarin and N-acetyl-L-cysteine are being already used in the clinical treatment of liver injury and they exhibit a potent hepatoprotective activity, but with certain limitations such as gastric irritation, allergies and limited efficacy (Park et al., 2004). This indicates that there is still the need of finding highly efficient and reliable drugs with insignificant side effects for the prevention of acute liver and renal failure.

Withania somnifera, a well-known Ayurvedic medicine, is known to exhibit antioxidant, anti-arthritis, anti-cancer, anti-inflammatory and anti-ageing properties (Rasool and Varalakshmi, 2006; Masour and Hafez, 2012). It has been observed that it can also ameliorate hepato and nephrotoxicity triggered by few compounds due to the manifestation of some of its effective elements. W. somnifera roots are proven to be greatly medicinally functional organ and demonstrates oxygen scavenging influences (Singh et al., 2010). Withaferin A (WA) belongs to the group of WTs and is present in W. somnifera roots. It is the major therapeutically active and is highly oxygenated (Berghe et al., 2012). It is also known to inhibit the activity of CYP enzymes which are mainly responsible for the bioactivation of xenobiotics (Dey et al., 2015). Considering these indications of pro-oxidant, CYP inhibiting and anti-inflammatory properties of W. somnifera, the investigation to assess
the ameliorative effect of *W. somnifera* and its most therapeutic constituent, Withaferin A on BB induced hepatic and renal damage in selected animal models was performed. There are no biologically significant changes in the metabolism and toxicity profile of bromobenzene with respect to the gender or the animal model of mice or rat used; therefore, this study was performed regardless of the sex and both the animal model of mice and rat were studied. Further, *W. somnifera* was initially used for the primary studies and since it was found to be effective, therefore, based on the docking analysis its most therapeutically active component Withaferin A was further analysed for its protective effects. Also, molecular analysis was done to show the interactions between selected important CYP enzymes and nuclear receptors with Withaferin A to study its mechanism of action for prevention of acute liver and renal injury.

Fig 1.7: Postulated method of action of *Withania somnifera*