Review of Literature
Metals are ubiquitous in our daily lives and it is not an exaggeration to say that ‘We live in a world of metals’. Beyond the use of metals in the products we create such as cars, planes and buildings, metals also play an important role in biological systems. All heavy metals are potentially toxic, yet many metals are essential for life. Certain heavy metals such as zinc, iron, magnesium etc., referred to as essential trace metals, are nutritionally essential for life processes, while others such as cadmium, lead etc. are harmful to them.

Industrialization of the world has been accompanied by the extraction and distribution of mineral substances from the natural deposits. Metals continue to be heavily used in consumer products, industrial and military equipment resulting in continued environmental damage from mining and metal processing. Concern about the increasing environment levels of heavy metals has stirred interest in the study of effects of toxic substances on living systems. Cadmium is one such toxic environmental pollutant that serves no biological function.

The present study was envisaged with two-fold objectives: one being, the evaluation of acute and chronic Cd toxicity in mice liver and kidney, since these are the two primary target organs in which its toxic effects are expressed (Chwelatuik et al., 2006). The other aspect was to investigate the modulating role of curcumin pre-treatment on acute Cd exposure.

Curcumin is a phenolic compound derived from turmeric and believed to have a wide spectrum of bio-protective properties that have been reported only after its repeated administration for longer durations (a week or more). Till date, there is hardly any report on the effect of curcumin administration for short durations. Also the modulating role of curcumin treatment on Cd toxicity is scarcely reported. Thus, keeping in mind the shortcomings of the reports on Cd and/or curcumin, this study was undertaken. Henceforth, the review has been divided into 2 sections:

(A) Cadmium
(B) Curcumin

Heavy Metal Toxicants

The term toxicant refers to substances produced by or is a byproduct of anthropogenic (man-made) activities, whereas toxin refers to toxic substances that are produced by biological systems such as plants, animals, fungi or bacteria. Living organisms have been exposed to heavy metal toxicants for an immeasurable period of time. The industrialization of the world has dramatically increased the overall environmental
“load” of heavy metal toxins to the point that our societies are dependent upon them for proper functioning. The biomagnification of these toxicants in the living organisms is a matter of great concern to the present research scenario.

"Heavy metals" are chemical elements that have a specific gravity 5 times or more than that of water (1 at 4°C). Some well-known toxic metallic elements with a specific gravity > 5 are arsenic (5.7), cadmium (8.65), lead (11.34) and mercury (13.546) (Lide 1992). Heavy metal toxicants contribute to a variety of adverse health effects that include behavioural, physiological and cognitive changes in an exposed individual (Chwelatiuk et al., 2006). They are systemic toxicants with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects and can induce impairment and dysfunction of the eliminative organs (colon, liver, kidneys, skin), enzymatic and energy production pathways, blood, cardiovascular, endocrine (hormonal), gastrointestinal, immune, nervous (central and peripheral), reproductive and urinary systems. The degree to which a system, organ, tissue or cell is affected by a toxic heavy metal depends on the toxicant itself and the individual's degree of exposure (Dudley et al., 1985; Goyer and Cherian, 1995; Shaikh et al., 1999).

Heavy metal toxicity represents an uncommon, yet clinically significant medical condition. If unrecognized or inappropriately treated, it can result in significant morbidity and mortality. The most common heavy metals implicated in acute and chronic conditions include cadmium, chromium, lead and arsenic that constitute a very frequently occurring quaternary mixture at hazardous waste sites (ASTDR, 2003).

**Cadmium**

Cadmium (Cd) is today regarded as the most serious contaminant of modern age (Auroville Innovative Urban Management, 2003). Friedrich Stromeyer first discovered Cd in Germany in 1817. It is a group II B metal that has an atomic weight of 112.41 g/mol and exists in the 0 and +2 oxidation state. The name is derived from the Latin cadmia and the Greek kadmeia. It is a naturally occurring metallic element that is a component in the earth’s crust and present everywhere in our environment (Table 2.1).

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<td>Atmosphere</td>
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<td>Earth’s crust</td>
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<td>Marine sediment</td>
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Industrial applications for Cd were first developed in the late 19th and early 20th century. Cd-sulfide based pigments were used as early as 1850 and appeared prominently in
the paintings of Vincent Van Gogh. Thomas A. Edison invented Ni-Cd batteries in the early parts of the 20th century. The early significant industrial use of Cd, however, was in Cd coating for the corrosion protection of steel (IARC, 1993; Llewellyn, 1994).

Pure Cd is a soft, silver-white metal. However, it is usually not found in the environment as a metal, but as water-soluble components like cadmium oxide, cadmium chloride, cadmium sulfate or cadmium sulfide (Zalups and Ahmad, 2003).

A ranking and total hazard score for approximately 400 substances was given in NPI reporting list (2005) based on human health and environmental hazards.

- Cd with its compounds was placed on the 6th rank.
- The total hazard score taking into account both human health and environmental criteria was 4.3.
- On a health hazard and environmental rating of 0-3, Cd and its compounds register 2.3 and 2 respectively (score of 3 represents a very high hazard, 2 represents a medium hazard, 1 is harmful to health and 0 a negligible hazard) (NPI cadmium compounds fact sheet, 2005).

Environmental Contamination by Cd

The soil, air and water contamination by Cd results mainly from natural processes and anthropogenic activities (Devi et al., 2001). Nearly 25,000 tons of Cd is pumped into the environment each year. About half of this Cd is released into environment from gradual natural phenomena, such as rock erosion, abrasion, forest fires and volcanic eruptions. Cd is naturally present in air, water, soil and foodstuffs. The rest of the Cd is released through human activities, such as mining, manufacturing, burning household/industrial waste, fossil fuels (such as coal or oil), incineration of municipal waste materials and production of artificial phosphate.
fertilizers. Cd may also escape into the air from zinc, lead or copper smelters (Fig. 2.1).

**Human Exposures to Cd**

Cadmium due to its unique properties such as great resistance to corrosion, low melting point, excellent electrical conduction, resistance to chemicals and high temperatures is extensively used in many important industrial applications (IARC, 1993). It is widely used in electroplating (cadmium oxide and cadmium sulfate), special alloys (a constituent of easily fusible alloy), pigments (cadmium sulfide), coatings, plastics stabilizers (cadmium stearate) and above all (almost 70% of its use), in rechargeable Ni-Cd batteries (cadmium oxide) which account for significant industrial/occupational exposure. Cd compounds are also used in printing, textiles, television phosphors, photography, lasers, semiconductors, pyrotechnics, solar cells, scintillation counters, as a neutron absorber in nuclear reactors, in dental amalgams, manufacture of fluorescent lamps, engraving, automobile and aircraft industries, as pesticides and polymerization catalysts. Cd is also found in superphosphate fertilizers. Cd pigments also produce intense colourings such as yellow, orange and red, and are well known pigments in artists' colours, plastics, glasses, ceramics and enamels (Stokinger, 1981; Friberg and Elinder, 1983).

**Accepted Exposure Doses**

Over the past 20 years, human exposure to Cd has continued to decrease. World Health Organisation (WHO) has approved Provisional Tolerable weekly Cd intake to be 7 μg/kg bw (WHO, 2000).

**Exposure Pathway**

Cadmium enters the air and spreads with the wind finally settling onto the ground or surface water as dust. It is strongly adsorbed to organic matter in soils and can be extremely dangerous as the uptake through food increases. This is a potential danger to animals that are dependent upon plants for survival and subsequent toxicity, since Cd does not break down and can bio-accumulate over time finally resulting in biomagnification in animal tissues (NRC, 2000).

A well-established example of Cd poisoning was observed in the Fuchu area of Japan and caused a disease called *Itai-Itai* (‘ouch-ouch’) which was characterized by
severe kidney and bone diseases. Unknowingly, the rice consumed had been grown in paddies of areas where mining wastes rich in Cd had been dumped. The estimated Cd intake of Itai-Itai victims was found to be 1 mg/day (Prasad, 2004), which was 20 times higher than the maximum permissible limit and 200 times more than the normal intake in unexposed populations.

During the past decade, regulatory pressure to reduce or even eliminate the use of Cd has gained momentum in many countries. Cd is one of the eleven metals on the U.S. Environmental Protection Agency (EPA) list of persistent and bio-accumulative toxic pollutants. Therefore, the EPA targeted a 50% reduction in its use in United States by 2005. The European Union is evaluating a proposal to ban all Ni-Cd batteries containing more than 0.002% Cd beginning on January 1, 2008, and to increase the collection rate for all spent industrial and automotive batteries (Plachy, 2005).

Bio-availability and Bio-accumulation

Irrespective of its route of entry into the body, a chemical must be bio-available in order to be absorbed into the blood stream. It is then distributed throughout the body, entering various organs where it can either be bio-transformed (metabolized) and then excreted or bioaccumulated in the liver, kidney and other soft tissues as in the case of Cd bound to the protein metallothionein (MT) (Masters et al., 1994).

Absorption, Distribution, Metabolism and Excretion (ADME)

In mammals, Cd is virtually absent at birth, but accumulated with time, especially in the liver and kidneys. Rapid renal concentration occurred mainly during the early years of life (Henke et al., 1970). Accumulation in the kidneys continued up to 50–60 years of age and fell thereafter, possibly due to age-related changes in kidney function. 50–75% of the total body burden of Cd was found in liver and kidney (Friberg et al., 1985). Its concentrations may fall subsequent to renal damage with increased leakage of bound Cd into the urine (Nomiyama et al., 1982).

The accumulation of Cd in the liver and its subsequent redistribution to the kidney was due to efficient MT synthesis in the liver; Cd–MT was slowly released into the plasma, filtered through the glomeruli and reabsorbed in the proximal tubules. After exposure to normal dietary concentrations of Cd (10–30 μg/day), about 50% of the body burden was found in kidneys, 15% in the liver and 20% in muscle (Kjellstrom, 1979). Lower concentrations were found in brain, bone and fat (Cherry, 1981).
(i) Absorption

Humans are generally exposed to Cd by two main routes - inhalation and ingestion. Cd enters the blood by absorption from stomach or intestines after ingestion of contaminated food or water or by absorption from the lungs. The absorption of Cd into blood from the gastrointestinal tract was low (only about 5%) (ATSDR, 2003), whereas, it was readily absorbed by inhalation (30 to 50%) (Moore, 1971). Absorption by skin was relatively insignificant, although small amounts of Cd could be absorbed percutaneously during long periods of exposure (Wester et al., 1992).

The amount of Cd needed to cause an adverse effect in an exposed person depends on the chemical and physical form of the element. In general, Cd compounds that dissolve easily in water (e.g., cadmium chloride), or the body (e.g., cadmium oxide) tend to be more toxic than the less soluble ones (e.g., cadmium sulfide).

The factors that affect the absorption of Cd include the animal species, gender, type of compound, dose, frequency of administration, age or stage of development, pregnancy and lactation, presence or absence of drugs, nutritional status and interactions with various nutrients such as metal ions and chelators (Taguchi and Suzuki, 1981; Rummler et al., 1989; Groten et al., 1991; Min et al., 1992; Ehsenhans et al., 1997). In mice, the degree of absorption depended on the age of the animal: young mice retained 10%, while adult mice retained only 1% (Matsusaka et al., 1972). A similar difference in absorption with age was found in neonatal mice, which absorbed Cd to a much greater extent than adult ones (Engstrom and Nordberg, 1979).

Low dietary concentrations of Ca and protein promoted absorption of Cd from the intestinal tract of experimental animals (Friberg et al., 1975). A low Zn and Fe status in laboratory animals and humans have also been shown to result in greater absorption of Cd (Hamilton and Valberg, 1974; Flanagan et al., 1978; Flora et al., 1998).

(ii) Distribution/Transport

Once absorbed, Cd was transported in the blood, mainly in erythrocytes and was intracellularly bound to low and high molecular mass protein fractions (Nordberg, 1972; Goyer, 1991). Normal blood Cd levels in adults are < 1µg/dl. The fraction with a low relative molecular mass was similar to metallothionein (MT). Plasma MT has an important role in the transport of Cd. It could contain up to 11% of Cd by weight, bound to sulphydryl groups (Elinder and Nordberg, 1985), and occurred in large quantities in the liver, particularly after exposure to Cd.
A large number of animal studies, including those on dogs showed that immediately after parenteral administration, maximum load of Cd was present in the plasma (Walsh and Burch, 1959; Friberg et al., 1974). Plasma concentrations decreased rapidly during the first hours of injection, subsequently reaching a level, which was less than 1% of the initial level within 24 hours and after that the level decreased slowly. During the initial period of Cd toxicity which is known as fast-elimination phase, Cd in mouse plasma was bound to plasma proteins with a molecular weight of 40,000 to 60,000 (probably albumin), whereas in the subsequent phase (more than 24 hours after injection), it was partly bound to a low molecular weight (LMW) protein of the same size as MT (Nordberg, 1978).

The route of administration was shown to be an important variable affecting the distribution of Cd. The higher the intensity of Cd exposure, the higher was the initial liver-to-kidney concentration ratio (Zalups and Ahmad, 2003). Cd bio-accumulated in kidney upto 8 months after exposure and then exceeded the levels in liver (Gunn and Gould, 1957). The pancreas and spleen also showed relatively high concentrations (Nordberg and Nishiyama, 1972). The levels of Cd in liver increased rapidly following acute exposures whereas, it accumulated in kidney after chronic exposures.

While Cd could reach the embryo or foetus early in gestation, little transfer occurred across the fully developed placenta (Ahokas and Dilts, 1979). The placenta retained Cd after its exposure to low concentrations due to induction of MT in it. Essential elements like Zn and Cu were also bound to MT in the placenta (other than Cd), but for reasons not known, only the essential metals were transported to the foetus (Goyer, 1986). The concentrations of Cd in the organs of embryos, fetuses and neonates were three orders of magnitude lower than the corresponding concentrations in adult women (Chaube et al., 1973; ATSDR, 1989).

**Role of Metallothionein (MT) in Transport, Metabolism and Toxicity of Cd**

**Nature and Production**

Metallothionein (MT) is a metal-binding protein of low molecular weight, which has a key role in the metabolism of Cd. It is rich in cysteine that are involved in binding of the metals, but contains no aromatic amino acids or histidine (Kagi and Vallee, 1960, 1961). It was identified by Margoshes and Vallee (1957) in cortex of horse kidney. Its molecular weight is about 6,600 Da (6,000 Da for the apoprotein moiety, thionein) with a non-globular shape and an irregular secondary structure. They all have a
dumbbell-like shape with two separate protein domains containing in their core mineral-like clusters built up of several tetrahedral Me(II)-Cys units.

Generally, two major forms of MTs are reported to be present in mammalian tissues, particularly liver and kidney, i.e., MT-I and MT-II. Induction of synthesis was reported to be under the control of a large group of genes and was stimulated by glucocorticoids and the essential metals like Zn and Cu, as well as by the toxic metals Cd and Hg (Karin et al., 1981; Karin and Richards, 1982). MT may bind seven metal ions per molecule between two separate metal-cysteine clusters and a single molecule may contain more than one metal, e.g., Cd, Zn, Hg and Cu.

Role of MT in Cd Bio-transformation and Bio-accumulation

It has been demonstrated in animal and in vitro tissue studies that MT provided a protective role against Cd toxicity (Cherian and Nordberg, 1983). Mice pre-treated with Cd had increased tolerance to subsequent Cd exposure (Nordberg et al., 1971) and might protect them from subsequent Hg toxicity (Piotrowski et al., 1974). Piscator (1964) suggested that some of the Cd-binding MT in the liver might migrate into the blood stream which was quickly cleared by glomerular filtration and reabsorbed in the renal tubules or excreted in the urine (Fowler and Nordberg, 1978). If the Cd-MT concentration was low in the plasma, then the tubular reabsorption was complete, but in the presence of high concentrations, uptake in the tubular cells from the tubular fluid was saturated (Nomiyama and Foulkes, 1977; Foulkes, 1982).

Further studies suggested that intraperitoneally administered Cd-MT entered proximal renal tubular lining cells in pinocytotic vesicles and fused with lysosomes (Fowler and Nordberg, 1978; Squibb et al., 1982). The MT on degradation, released Cd into the cytosol and resulted in cellular degeneration and necrosis within 8-24 hours. Ohta et al. (2000) suggested that the pathogenesis of renal tubular cell toxicity was related to non-MT-bound Cd, which was rapidly bonded to existing MT sites or induced the synthesis of new MT.

The prevalence of nephrotoxicity rather than hepatotoxicity in chronic Cd exposures has been suggested to be due to several factors. Firstly, the release of hepatic Cd-MT or its presence in the blood could result in preferential bio-accumulation of Cd in the kidneys. Secondly, it was shown that in experimental animals kidneys might accumulate MT mRNA in response to Cd exposure to only about half the level of the liver (Koropatnick and Cherian, 1988). Thus, the kidney
may not be able to synthesize MT as efficiently as the liver in response to Cd exposure, resulting in bio-accumulation of non-MT Cd in the kidney, but not in the liver.

**Hepatic Handling of Cd**

The liver bio-accumulated more Cd than any other organ, especially following acute exposures to inorganic salts of Cd (Liu et al., 2001; Zalups and Barfuss, 2002). The Cd in the blood was bound to plasma proteins, such as albumin and ferritin (Brown and Shockley, 1982; Nordberg and Nordberg, 1988) or in the form of conjugates of cysteine (Cys), GSH and other low-molecular-weight thiols (R-Cys-R) (Lash and Jones, 1985). Receptor-mediated endocytosis of one or more protein conjugates of Cd, by proton-coupled divalent metal transporter (DMTI) (Trinder et al., 2000) or metal transporter protein I (MTPI) (Abboal and Haile, 2000), both localized on the sinusoidal plasma membrane, may be an important mechanism by which Cd was taken up by the hepatocytes (Oka et al., 1989). The organic anion/cation and amino acid transporters may also be involved in uptake of Cd from the sinusoidal blood (Grundemann et al., 1994; Jacquemin et al., 1994; Kullak-Ublick et al., 1994) or it may "mimic" Ca and enter the hepatocytes through Ca channels (Blazka and Shaikh, 1991; Souza et al., 1997).

Goering et al. (1985) reported that once Cd entered into the hepatocytes, it became a part of exchangeable and non-exchangeable intracellular Cd, capable of inducing transcription of the genes for MT-I and MT-II. MT in hepatocytes resulted in the formation of Cd-MT complex that inhibited the direct interactions between Cd ions (Cd$^{2+}$) and critical nucleophilic sites within the cells (Goering and Klaassen, 1984). However, if the intracellular exchangeable Cd increased beyond the protective mechanism of the hepatocytes, oxidative stress was induced leading to lipid peroxidation in the plasma membrane and resulted in cell death by necrosis or apoptosis (Sarkar et al., 1995; Stohs and Bagchi, 1995; Shaikh et al., 1999; Rikans and Yamano, 2000; Stohs et al., 2001). As hepatocytes underwent cell death, they released Cd-MT into the sinusoids and canaliculi, which was carried to the kidneys, filtered freely at the glomerular filtration barrier and may be taken up by proximal tubular epithelial cells (Zalups et al., 1992).

Cadmium ions entering the hepatocytes interacted with the intracellular GSH to form an S-conjugate of GSH (Lash et al., 1995; Zalups, 1999) and were transported
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into the biliary canaliculi by multiple drug-resistance protein 2 (MRP2) and/or a GSH-transporter (Ballatori and Dutczak, 1994). The GSH S-conjugates of Cd were rapidly broken down to the corresponding Cys S-conjugates by actions of \( \gamma \)-glutamyltransferase and cysteinylglycinase located on the canalicular plasma membrane (Zalups and Ahmad, 2003).

Renal Handling of Cd

The chemical derivatives of Cd filtered at the glomerulus are not known and they may either be filtered into the proximal tubular lumen or be involved in the luminal absorptive transport of Cd (Endo, 2002). Experimental findings of Cannon et al. (2001) with mercuric conjugates of GSH indicated that GSH S-conjugates (GSH-Cd-GSH) of Cd filtered into the proximal tubular lumen were rapidly degraded to cysteinylglycine S-conjugates (Cys-Gly-Cd-Cys-Gly) and Cys S-conjugate of Cd (Cys-Cd-Cys) by brush-border enzymes \( \gamma \)-glutamyltransferase and cysteinylglycinase, respectively) (Zalups, 2000b). These Cys S-conjugates may subsequently, enter the proximal tubular epithelial cells via amino transporters by acting as "mimics" of amino acids cysteine/cysteine or through MRP2, expressed in the luminal plasma membrane of proximal tubular epithelial cells (Schaub et al., 1999).

Calcium channels may also be involved in Cd uptake at the luminal membrane of proximal tubular epithelial cells. It has been hypothesized that Cd\(^{2+} \) may be delivered to the binding sites of Ca channels in the form of thiol- or protein-conjugates, where these could undergo a ligand-exchange reaction (Endo, 2002). Support for this hypothesis came from experiments showing that Cd could disrupt the intercellular junctions of an immortalized line of cultured porcine proximal tubular epithelial (LLC-PK\(_1\)) cells by binding to the extracellular Ca-binding sites of the E-cadherin/catenin network of the zonula adherens, which was an integral component of the junctional complex (Endo et al., 1998, 1999). Zalups (2000a) indicated that when Cd was co-administered to rats with GSH or Cys, there was a significant increase in its renal basolateral uptake and accumulation due to these carrier systems.

Increased expression of renal MT genes (MT-I and MT-II) and especially those of the proximal tubular epithelial cells was observed following Cd exposures. If the intracellular exchangeable Cd increased beyond the proximal tubular epithelial cell’s protective mechanism, then oxidative stress was induced. This may either alter
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mitochondrial respiratory activity and lead to lipid peroxidation of plasma membrane or cause perturbations in cellular metabolism finally leading to cell death by necrosis or apoptosis, thereby, releasing Cd (Dudley et al., 1985; Zalups and Ahmad, 2003).

iii) Metabolism

There is no reported direct metabolic conversion of Cd and it binds to various biological components, such as proteins, non-protein sulphhydril and anionic groups of various macromolecules (ATSDR, 1989). MT binds Cd and some other metals, thereby, playing an important role in determining the bio-accumulation of Cd in the body (e.g., concentration of Cd in the kidneys) (Ohta et al., 2000; Zalups and Ahmad, 2003).

iv) Excretion

The principal route of excretion is by urine in which normally a small amount of Cd is excreted (ATSDR, 1989). With increased bio-accumulation of Cd, renal dysfunction developed, that further resulted in excretion of hepatic and renal Cd deposits (Nordberg and Piscator, 1972; Nomiyama and Nomiyama, 1976). The mechanism of faecal excretion of Cd was thought to involve both sloughed mucosal cells and biliary excretion. After exposure to low or moderate Cd doses, the amount excreted in the faeces and urine was the same. Minor routes of excretion included hair, breast milk, and pancreatic fluid, but collectively these routes made little contribution to the total excretion or biological half-time of Cd in the body. This slow excretion of Cd, resulted in extremely long biological half-times in animals (200 days to 22 years) (Friberg et al., 1985).

TOXICOLOGY

Toxicology is the study of the adverse effects (cellular, biochemical and molecular) of chemicals on living organisms and assesses the probability of their occurrence. A chemical agent does not produce toxic effects in a biological system unless that agent or its metabolic breakdown (bio-transformation) product reaches appropriate sites in the body at a concentration and for a sufficient length of time adequate to produce a toxic manifestation. Many chemicals are of relatively low toxicity in the native form, but when acted on by enzymes in the body, they are converted to intermediate forms that interfere with normal cellular biochemistry and physiology.
Cadmium - Mode of Action

While the importance of Cd as an environmental health problem has long been recognized, relatively little is known about the specific mechanisms by which Cd produces its toxic effects (Prozialeck, 2000). Free Cd ions (Cd^{2+}) in the cells initiate the synthesis of new MT, which binds the Cd, thereby, protecting the cell from the highly toxic Cd^{2+}. Toxicity may be considered to occur when the binding capacity of MT is surpassed (Chwelatuik et al., 2006).

The cellular and molecular mechanism of Cd toxicity have been investigated and Massaro (1997) broadly divided these into four groups:

(i) Interaction with signal transduction pathways (i.e., receptors, G-proteins, ion channels and kinases/phosphatases).

(ii) Genotoxicity (by induction of chromosomal alterations, causing single or double strand breaks, frameshift or missense mutations, inhibition of DNA repair and effects on gene regulation).

(iii) Cell death i.e., apoptosis and necrosis resulting from oxidative stress, altered intracellular ion homeostasis, increased cellular Ca level, mitochondrial dysfunction and ATP depletion.

(iv) Alterations in anti-oxidant systems namely, anti-oxidant enzymes, cellular glutathione levels, induction of heat shock proteins (HSP) and metallothionein (MT).

Effects of Cd on Gene Regulation

- Metallothionein (MT) and glutathione (GSH) - The most dramatic regulatory effect of Cd was its capability to induce the expression of genes for the synthesis of MT (Palmiter, 1994) and GSH (Hatcher et al., 1995) at concentrations of a few μM/l.

- Stress proteins - At elevated cytotoxic concentrations (200 μM), Cd induced a series of stress proteins.

  - Heat shock protein (HSP): HSP70, HSP90, and HSP110 were induced in the livers of rats treated intraperitoneally with CdCl₂ (Goering et al., 1993). Cd²⁺ evoked the synthesis of HSP25, HSP35, HSP70, HSP100 in chicken embryo cells (Levinson et al., 1980) and HSP60 in human hepatoma cells
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(Hiranuma et al., 1993).

- **Acute-phase reactants (APR):** These are proteins that protect cells from damage and consist of α1-glycoprotein and serum amyloid. These were found to be induced in rats after intraperitoneal injection of Cd or during inflammatory reactions (Prozialeck, 2000; Zalups and Ahmed, 2003).

- **Proto-oncogenes** - Non-cytotoxic doses of Cd have been shown to induce cellular proto-oncogenes - c-jun, c-fos and c-myc, also termed as immediate early or primary response genes and some other genes related to mitogenic stimulation (Hechtenberg et al., 1996). The induction of these genes indicated a mechanism by which Cd may promote the development of cancer and seemed to be independent of the mitogen-activated protein (MAP) kinase system (Schafer, 1997).

- **Tumour suppressor gene** - The induction of tumour suppressor gene p53 was also detected in Cd-treated mice (Zheng et al., 1996).

- **Cytokines** - Cd affected genes related to inflammatory responses. The transcription of the genes for the cytokines IL-1α, IL-1β, IL-6, ICAM-1, MIP-2 and TNF-2α as well as the secretion of these proteins was increased after CdCl₂ was given orally to mice during inflammatory processes (Kayama et al., 1995 a and b).

- **gadd 153 gene** - The product of this gene serves an emergency function to protect cells from proliferation in the presence of damaged DNA. CdCl₂ was shown to affect the induction and expression of this gene in HeLa cells at very high, cytotoxic concentration of 200 μM (Luethy and Holbrook, 1992).

**Exposure, Prognosis and Treatment**

For characterizing the toxicity of a chemical, information is needed about the effects following both acute and chronic exposure.

**Acute Exposure**

Acute exposure refers to single administration or repeated exposures to a chemical for less than 24 hours. Acute exposures to agents that are rapidly absorbed are likely to produce immediate toxic effects but can also produce delayed toxicity.

Ingestion of Cd salts affected the gastrointestinal tract, liver, kidneys, nervous and
cardiovascular systems (Bernard and Lauwerys, 1986a; Ellenhorn and Barceloux, 1988), while, inhalation of Cd-containing fumes or dust affected the respiratory tract and lungs causing bronchitis, pulmonary oedema and interstitial pneumonia (Beton et al., 1966; Barnhart and Rosenstock, 1984; Buckler et al., 1986). Although dermal absorption of Cd was not significant, skin irritation may be caused by some Cd compounds (Sittig, 1985; Lenga, 1988), while Cd dust and fumes were likely to produce corrosive damages of the eyes (Lenga, 1988).

Biomedical analysis - In cases of acute poisoning, cardiovascular parameters (heart rate, blood pressure), cardio-respiratory function (chest X-ray, ECG, blood gases) and electrolyte balance may be monitored.

Antidote treatment - It has been recommended that routinely used calcium disodium EDTA and dimercaprol (BAL) should not be used in cases of acute Cd poisoning because of their nephrotoxic potential (Friberg and Elinder, 1983; Ellenhorn and Barceloux, 1988). Studies in rodents showed that, for acute oral Cd intoxication, meso-2,3-dimercaptosuccinic acid given orally (Basinger et al., 1988; Andersen and Nielsen, 1988; Andersen, 1989) or calcium disodium diethylenetriaminepentaacetate (DTPA) given parenterally (Andersen, 1989) were the most effective antidotes, provided the treatment was started soon after Cd ingestion.

Chronic Exposure

Chronic exposure refers to long-term exposure to lower doses of a chemical. Chronic exposure to a toxic agent may produce some immediate (acute) effects after each administration, in addition to the long-term, low level or chronic effects of the toxic substance.

Ingestion of Cd salts affected the kidneys and lungs resulting in proteinuria (Tsuchiya, 1967) and impairment of lung function (Sittig, 1985; Goyer, 1986), respectively. Similarly, inhalation of Cd-containing fumes or dust also affected the kidneys and bone causing proteinuria, renal stones (Friberg and Elinder, 1983) and a severe bone disease-Itai-itai.

In Cd-polluted areas of Japan, such as the Jintzu river basin, where high concentrations of Cd had been discovered in rice, signs of renal damage, such as proteinuria, glycosuria and β2-microglobulinuria were observed in the general population, similar to those seen in industrial workers exposed to Cd (Kjellstrom et al., 1977b; Kojima et al., 1977; Shiroishi et al., 1977). Bone lesions were usually a
late manifestation of severe chronic Cd poisoning and characterized by osteomalacia, osteoporosis and spontaneous fractures (Bernard and Lauwerys, 1986b; Hallenbeck, 1986).

**Biomedical analysis** - In chronic poisoning, early nephrotoxic effects of Cd could be detected on the basis of the measurement of urinary proteins, which reflected the functional integrity of the tubule or the glomerulus. Low molecular weight proteins such as β2-microglobulin, retinol-binding protein, or a1-microglobulin were used for screening proximal tubular injury, whereas the analysis of urinary high molecular weight proteins such as albumin, permitted the assessment of glomerular filtration selectivity. The urinary activity of α-N-acetylglucosaminidase was also a sensitive indicator of excessive absorption of Cd.

**Antidote treatment** - There is no current consensus on the recommended chelation treatment for chronic Cd exposure. Jones and Cherian (1990) reported reduction of body, renal and hepatic levels of Cd in mice treated with sodium N-(4-methoxybenzyl)-D-glucamine dithiocarbamate.

**Cadmium – A Systemic and Cumulative Poison**

Most chemicals that produce systemic toxicity do not cause a similar degree of toxicity in all organs, rather drastic toxicity in one or two organs is observed. Systemic effects require absorption and distribution of a toxicant from its entry point to a distant site, at which deleterious effects are produced. These sites are referred to as the target organs of toxicity of a particular chemical and often may not be the site of the highest accumulation of the chemical. Cd is a cumulative poison i.e., danger lies primarily in the regular consumption of foodstuffs with low contamination (Auroville Innovative Urban Management, 2003) and caused damage to various organs including the lung, liver, kidney, pancreas, testis, bone and placenta (ATSDR, 1999).

**Effects of Cd on Liver**

The liver plays a critical role in metabolizing foreign substances including nutrients, therapeutic drugs and environmental toxicants, some of which can have an adverse effect on it. Cd is a well-established potent hepatotoxicant and the liver is the most important target in acute Cd intoxication (Horiguchi et al., 2001).

Histological evaluation of liver injury by Dudley et al. (1984) in Sprague-Dawley rats revealed that acute Cd toxicity comprised of hepatocellular swelling, sinusoidal
congestion, pycnosis and karyorrhexis. Time-course analysis of transmission electron photomicrographs suggested mitochondrial swelling, appearances of fibrillar material within the cytoplasm, changes in rough endoplasmic reticulum (RER) and nucleus (Dudley et al., 1984). These cellular changes may result in both apoptosis and necrosis (Habeebu et al., 1998).

**Acute Exposure In Vivo Studies:**
Buckler et al. (1986) reported focal hepatic necrosis after autopsy of a young woman who had ingested 150 g of CdCl₂, while fatty infiltration and acute centrilobular necrosis of the liver were observed in a patient who had died following inhalation of Cd fumes (Taylor et al., 1984).

**Chronic Exposure In Vivo Studies:**
Friberg (1950) demonstrated fibrotic changes in the liver of rabbits exposed to repeated subcutaneous injections of Cd. Periportal and interlobular collagen deposition was found in the liver of rabbits exposed to drinking water containing 160 mg Cd/l for 6 months (Stowe et al., 1972). After i.p. injection of Cd (upto 1.25 mg/kg bw for 6 weeks), decreased glycogen content and increased activity of hepatic gluconeogenic enzymes in rat were reported by Merali et al. (1974) and Chapatwala et al. (1982). In long-term studies, rabbits exposed to 300 mg Cd/kg diet for 54 weeks showed amyloid deposits in the liver (Kawai et al., 1976), while Rhesus monkeys exposed to the same dose for 12 weeks developed increased levels of plasma enzymes (GOT, GPT, and LDH) (Nomiyama et al., 1979).

**Effects of Cd on Renal System**
The kidney is the critical organ of Cd toxicity in humans and other mammals exposed for long periods to the relatively small amounts of Cd that might be present in foods. Many studies in experimental animals have demonstrated an association between morphological and/or functional changes in the kidney and the renal concentration of Cd (WHO 1992).

Initial morphological changes observed post-Cd exposures were degeneration, regeneration, eosinophilic bodies, vacuolization and basophilic changes, that were limited to proximal tubular epithelial cells (Ohta et al., 2000). These were followed by cellular atrophy, interstitial fibrosis, pathological changes in mesengial cells, glomerular sclerosis and increased thickening of the glomerular basement membrane.
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(Stowe et al., 1972; Chwelatiuk et al., 2006). These morphological changes were also associated with biochemical evidence of renal tubular dysfunction, i.e., presence of proteins of low relative molecular mass in the urine (β2-microglobulin and retinol-binding protein), glucosuria, aminoaciduria, hypercalciuria, enzymuria (N-acetyl-β-glucosaminidase) and high urinary concentration of Cd and MT (WHO, 1992; ATSDR, 1999). Uriu et al. (1998) found a decline in the glomerular filtration rate in rats treated parenterally with CdCl₂, which was associated with a lowered filtration fraction.

Various hypotheses have been proposed to explain the nephrotoxicity of Cd and particularly the role of MT, a metal-binding protein. Ohta et al. (2000) suggested that renal-cell injury occurred when the critical concentration of Cd exceeded MT’s binding capacity. MT protected against Cd toxicity by binding to it and forming Cd–MT complex that was non-toxic when stored within cells in experimental animals and in vitro. Goyer et al. (1989) suggested that an unidentified form of Cd which was not bound to MT might become available resulting in nephrotoxicity.

Acute Exposure In Vivo Studies:

Ingestion and inhalation of Cd resulted in oliguria, anuria, nocturia and acute renal failure in humans (Taylor et al., 1984; Bernard and Lauwerys, 1986a; Buckler et al., 1986).

Chronic Exposure In Vivo Studies:

Long-term exposure to Cd resulted in renal tubular lesions with proteinuria, glucosuria, aminoaciduria and histopathological changes. Renal tubular dysfunction induced by low concentrations of Cd may progress to interstitial nephropathy with longer exposure (Aughey et al., 1984). The presence of proteins of relatively low molecular mass in the urine was a sensitive biomarker of renal tubular dysfunction, although hypercalciuria may be an equally sensitive biomarker. The critical concentration of Cd for the induction of nephropathy was 50–200 μg/g of renal cortex (Bernard et al., 1981; WHO, 1992).

The weight of the kidneys and the incidences of glomerular sclerosis, tubular-cell degeneration and interstitial fibrosis were found to be increased in rabbits fed a diet containing 160 mg CdCl₂/kg for 200 days (Stowe et al., 1972). Kajikawa et al. (1981) also reported morphological changes in the kidneys of rats exposed to 200 mg CdCl₂/l for 91 weeks via drinking water. Histologically, they observed degenerative changes
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in the proximal convoluted tubules, whereas electron microscopy revealed proliferation of smooth endoplasmic reticulum, vacuolization and coagulative necrosis of the tubular cells with no significant changes in the glomeruli or interstitial tissue.

Hiratsuba et al. (1999) suggested that the difference in the pattern of distribution of Cd in the liver and kidneys may be due to a difference in the form of absorbed Cd, i.e., free ion (Cd\(^{2+}\)) or Cd–MT complex or hyper-cadmiumuria due to renal tubular toxicity at higher Cd doses.

Other Systemic Effects of Cd

One of the major health effects of excess Cd in food was a severe bone disease known as Itai-Itai disease, characterized by osteoporosis/osteomalacia and associated with severe Cd nephropathy in which women were severely debilitated (Goyer et al., 1994). The populations known to be affected were exposed to excess Cd in rice. Most of the findings indicate that direct effects of Cd on bone mineralization developed after long-term Cd exposure, possibly related to calcium deficiency (Staessen et al., 1991) or an indirect effect on calcium absorption via vitamin D hydroxylation that occurred only following renal damage (Aoshima and Kasuya, 1991) leading to osteomalacia (Jarup et al., 1998; Alfven et al., 2000). Cd in bone may thus interfere with calcification, decalcification or bone remodeling (Kogan et al., 1972).

The blood–brain barrier and circumventricular epithelial cells having tight junctions limit the entrance of Cd into the central nervous system. The choroid plexus epithelium cells accumulated highly toxic metals from the blood or cerebrospinal fluid (Takeda et al., 1999). A variety of neurobehavioural (insomnia, confusion, restlessness) and biochemical (increase in the concentrations of nor-adrenaline, dopamine and impairment of enzymes involved in the synthesis of neurotransmitters) effects were produced on the nervous system of rodents repeatedly exposed to Cd (Beton et al., 1966; Taylor et al., 1984; Murphy, 1997).

The earliest symptom in cases of acute poisoning following inhalation, was slight irritation of the upper respiratory tract, followed by the development of an acute pneumonitis including cough, chest pain and dyspnoea, over the next few hours. Long-term occupational exposure to Cd dusts and fumes appeared to involve only mild obstructive lung disease (dyspnoea, reduced vital capacity and increased residual volume) and in some cases fibrosis with alveolar damage occurred (Edling et al., 1986).
Cardiovascular effects following Cd ingestion or inhalation included hypotension, dysrhythmias, shock and inflammatory changes of the myocardium (Buckler et al., 1986; Bernard and Lauwerys, 1986a). Chronic oral administration of Cd compounds to rats induced statistically significant elevation of blood pressure (Perry and Erlanger, 1974; Perry et al., 1977), which may be due to an increase in sodium and water retention (Nishiyama et al., 1986).

Hematological effects were reported following ingestion of CdCl₂, that included elevation in serum haemoglobin concentration, increased haematocrit and altered coagulation function (Buckler et al., 1986). Anaemia was a common finding in animals after both dietary (Wilson et al., 1941) and parenteral (Friberg, 1950) Cd exposures. Berlin and Friberg (1960) showed that Cd injections caused erythrocyte destruction while haemolytic anaemia in rabbits was reported by Axelsson and Piscator (1966). Decreased gastrointestinal absorption of iron due to Cd may be one mechanism for anaemia, which could be prevented by simultaneous feeding of iron or ascorbic acid (Fox and Fry, 1970; Fox et al., 1971).

Reproductive effects reported in experimental animals administered single injections of Cd salts included testicular necrosis due to endothelial damage (Gabbiani et al., 1974; Aoki and Hoffer, 1978; Francavilla et al., 1981) and resulted in permanent infertility (Barlow and Sullivan, 1982). Krasovkii et al. (1976) noticed decreased spermatozoa motility and spermatogenesis index in rats. A single injection of Cd salts that induced testicular haemorrhagic necrosis also induced haemorrhage and necrosis in the ovaries of rats (Kar et al., 1959; Parizek et al., 1968).

Gestational exposure to Cd (4.2 and 8.4 µg/ml in drinking water) resulted in decreased birth weight, retarded growth, delayed development of the sensory motor coordination reflexes, increased motor activity, developmental and behavioural defects with long-term implications on adult behaviour (Mohd et al., 1986). Fetal toxicity was observed when large doses of Cd salts given at later stages of pregnancy, were shown to induce severe placental damage and foetal deaths (Parizek, 1964; Chiquoine, 1965). Teratogenic effects such as exencephaly, hydrocephaly, cleft lips and palate, microphthalmia, micrognathia, clubfoot and dysplastic tail were observed when Cd doses close to the LD₅₀ were administered to pregnant females during early stages of gestation (Scharpf et al., 1972; Barr, 1973; Chernoff, 1973; Ishizu et al., 1973).

Cadmium affected the immune system after both acute and chronic exposures
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resulting in reduced T-lymphocyte dependent antibody production, lowered natural killer (NK) cell activity and suppressed macrophage activity (Stelzer and Pazdernik, 1983; Cook et al., 1984; Thomas and Imamura, 1986; Blakley and Tomar, 1986). Most of the studies examined immune function in young immunologically competent animals and these effects were considerably less marked in mature animals (Blakley, 1988).

Mutagenicity and Carcinogenicity

Cadmium compounds are weak mutagens and clastogens and ionic Cd (Cd\(^{2+}\)) is believed to be the active, genotoxic form (Beyersmann and Hartwig, 1994). At non-cytotoxic doses they inhibited DNA repair and detoxifying enzymes (Beyersmann and Hechtenberg, 1997) or stimulated the expression of various types of genes including cellular proto-oncogenes associated with enhanced cell proliferation (Harris, 1991), thereby, causing multi-organ cancers in rodents (Waalkes et al., 1992). In cultured mammalian cells, Cd compounds caused genetic damage, including gene mutations, DNA strand breaks, chromosomal damage, cell transformation and disrupted DNA repair (Devi et al., 2001).

Cadmium is reported to cause tumours of the testis, ventral prostate, haematopoietic system, i.e., leukemias, lymphomas and sacromas in rodents (IARC, 1993; Waalkes and Rehm, 1994). Waalkes et al. (1999) showed that chronic Cd exposure caused tumours of the adrenal gland and kidney, as well as proliferative, presumably pre-neoplastic lesions in the dorsolateral prostate in the Noble rat. Epidemiological studies have also linked Cd with cancer of the lungs (IARC, 1993), urinary bladder (Darewicz et al., 1998) and prostate (Brys et al., 1997) in humans. Accordingly, the WHO in 1993 classified Cd and its compounds as a category 1 carcinogens, based on epidemiological and toxicological studies in humans and experimental animals (IARC, 1993). However, the mechanism by which Cd induces carcinogenesis, is unknown (Hartman and Speit, 1994). One mechanism by which Cd was thought to induce tumours was the inhibition of DNA repair systems by displacing zinc from zinc-finger structures of DNA repair enzymes, leading to decreased removal of endogenous lesions (Hartwig, 1998).

Apoptosis or programmed cell death is a physiological and irreversible process in tissue homeostasis that leads to DNA fragmentation (Winter et al., 1998). It is primarily designed for elimination of unwanted and damaged cells without adversely
affecting the surrounding tissue and its morphological features are quite distinct from those of necrosis. These include chromatin margination along the nuclear membrane, nuclear condensation, budding, karyorrhexis, cell shrinkage and cell fragmentation (Winter et al., 1998). Although Cd is a powerful carcinogen, it might also induce apoptosis.

Habeebu et al. (1998) reported that Cd\textsuperscript{2+} induced apoptosis of hepatocytes in dose and time-dependent manner \textit{in vivo}. It was proposed that Cd\textsuperscript{2+} could induce apoptosis through different signaling pathways \textit{i.e}., the mitochondria- and the death receptor-dependent pathways. Apoptosis through the latter occurred when Cd\textsuperscript{2+} potentiated TNF-\alpha and TNF-related apoptosis, inducing ligand (TRAIL)-initiated cascade of events (Kim et al., 2002). Another possible mechanism involved, reactive oxygen species (ROS) formed under oxidative stress, which may induce apoptosis through pathways that recruit nucleus (Stohs et al., 2001), mitochondria (Halestrap et al., 2000) or both of them (Suisan et al., 1999). The ROS-damaged mitochondria released pro-apoptotic proteins (cytochrome c) into cytosol resulting in apoptosis (Stohs et al., 2001).

Piechotta et al. (1999) observed apoptosis as increased number of small DNA fragments in liver of \textit{dab} exposed to Cd. A progressive increase in the severity of necrosis, apoptotic and mitotic indices with increase in Cd dose was observed in mouse liver, which demonstrated that apoptosis was a major mode of acute hepatotoxicity in mouse, and preceded necrosis (Habeebu et al., 1998).

Curcumin - Primary Active Constituent of Turmeric

\textit{Curcuma longa}, a perennial herb, is a member of the \textit{Zingiberaceae} (ginger) family. The plant grows to a height of three to five feet and is extensively cultivated in Asia (India and China) and other countries with a tropical climate. It has oblong, pointed leaves and bears funnel-shaped yellow flowers (Dobelis, 1986).
The rhizome is the portion of plant used medicinally, which is usually boiled, cleaned, dried and crushed yielding a yellow powder. Dried *Curcuma longa* is the source of the spice turmeric, the ingredient that gives curry powder its characteristic yellow colour (Fig. 2.2). Turmeric is extensively used in foods for its flavour and colour. It has a long tradition of use in the Chinese and Ayurvedic systems of medicine, as an anti-inflammatory agent and for the treatment of flatulence, jaundice, menstrual difficulties, haematuria and haemorrhage.

The importance of turmeric in medicine took a new twist when it was discovered that the dried rhizome of *Curcuma longa* was very rich in phenolics, whose structures were identified as curcuminoids (Masuda et al., 1999). Curcuminoids are responsible for the yellow colour of turmeric and curry powder. They are derived from turmeric by ethanol extraction and are insoluble in water. They exhibit a variety of beneficial effects on health and on events that help in preventing onset and progression of certain diseases.

The best-researched active constituent of turmeric is curcumin (diferuloylmethane [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]). Its structure was first described in 1815 by Vogel and Pellatier and Lampe et al., (1913) proved its chemical identity (Joe et al., 2004). Curcumin has a unique conjugated structure that includes two methoxylated phenols and an enol form of a β-diketone (structure 1 in Fig. 2.3-A).
Curcumin comprises 0.3 to 5.4% of raw turmeric (Leung, 1980) and has been shown to have anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-viral and anti-infectious activities (Joe et al., 2004). It showed potent anti-oxidant activity against the oxidation of food components by its radical chain-breaking ability (Kunchandy and Rao, 1990; Masuda et al., 1993). Masuda et al. (2002) divided its anti-oxidation process into two stages, i.e., (i) Radical trapping stage and (ii) Radical termination stage.

The mechanisms of action for curcumin are not well understood and contradictory results have been reported. Some studies suggested that this natural compound possessed both pro- and anti-oxidative effects. Polasa et al. (2004) reported the anti-oxidant properties of curcumin in cellular experiments, where it suppressed the generation of reactive oxygen species (ROS) and protected against DNA damage induced by benz(a)pyrene or H$_2$O$_2$. On the other hand, curcumin may apparently act as a pro-oxidant. In human peripheral blood lymphocytes curcumin itself resulted in ROS that damaged DNA (Kelly et al., 2001). Recently Coa et al. (2006) reported that curcumin induced dose-dependent damage in both the mitochondrial and nuclear genomes in human hepatoma G2 (HepG2) cells and that the mitochondrial damage was more extensive. The cells were cultured in different mediums containing curcumin in the range 2.5 to 40 µg/ml. The lack of DNA damage at low doses suggested that low levels of curcumin did not induce DNA damage, but at high doses it imposed oxidative stress and damaged DNA. Their data reinforced the hypothesis that curcumin played a conflicting dual role.

**Pharmacokinetics**

Food additive curcumin is a non-toxic natural product (Commandeur and Vermeulen, 1996). Pharmacokinetic studies in animals demonstrated that 40-85% of an oral dose of curcumin passed through the gastrointestinal tract unchanged and most of the absorbed flavonoid was subsequently metabolized in the intestinal mucosa and liver (Wahlstrom and Blennow, 1978; Ravindranath and Chandrasekhar, 1980).

Ireson et al. (2001) reported that curcumin was first bio-transformed to dihydrocurcumin and tetrahydrocurcumin and these compounds were subsequently converted to monoglucuronide conjugates. Thus, curcumin-glucuronide, dihydro-curcumin-glucuronide, tetrahydrocurcumin-glucuronide, and hexa-hydrocurcumin (Fig. 2.3-B) were major metabolites in mice.
The systemic bio-availability of curcumin is low therefore, a part of its pharmacological activity may be mediated by itself or its related metabolites (Pan et al., 1999; Asai and Miyazawa, 2000). Pan et al. (1999) while investigating the pharmacokinetic properties of curcumin in mice following i.p. administration of curcumin (0.1 g/kg) observed that approximately 2.25 µg/ml of curcumin appeared in the plasma within the first 15 min. and one hour after administration, the levels of curcumin in the intestines, spleen, liver and kidneys were 177.04, 26.06, 26.90 and 7.51 µg/g, respectively with only traces (0.41 µg/g) present in the brain. Therefore, it was suggested that the bio-availability of curcumin, in the colon was greatest (Pan et al., 1999).

There is some evidence that orally administered curcumin accumulated in gastrointestinal tissues. When colorectal cancer patients were prescribed an oral dose of 3.6 g curcumin/day for 7 days prior to surgery, curcumin was detected in malignant and normal colorectal tissue (Garcea et al., 2005). In contrast, curcumin was not detected in the liver tissue of patients with liver metastasis of colorectal cancer after the same dose of oral curcumin, suggesting that oral curcumin administration may not effectively deliver curcumin to tissues outside the gastrointestinal tract (Ammon and Wahl, 1991; Garcea et al., 2004). Since the gastrointestinal tract seemed to be exposed more prominently to un-metabolized curcumin than any other tissue, their results support the clinical evaluation of curcumin as a colorectal cancer chemopreventive agent (Joe et al., 2001). A clinical trial conducted in the UK, found that plasma curcumin, curcumin sulfate and curcumin glucuronide concentrations were in the
range of 10 nM/l after one hour of an oral dose of 3.6 g curcumin, while curcumin and its metabolites could not be detected in plasma at doses lower than 3.6 g/day (Sharma et al., 2004).

It was interesting, however, to note that the bio-availability of curcumin was dramatically elevated by co-ingestion of piperine (a component of pepper) in both rats and humans (Shoba et al., 1998). Thus, the beneficial health effects of curcumin may be further magnified as a mixture of dietary additives are abundantly consumed as part of Asian diets (Bradlow et al., 1999).

Biological Activities

Curcumin has been claimed to represent a phytonutrient with bioprotective properties such as a potential anti-oxidant, anti-inflammatory and anti-cancer agent (Krishnaswamy et al., 1998; Li and Lin-Shia 2001; Balasubramanyam et al., 2003).

1. Anti-oxidant Activity

The discovery of anti-oxidant properties of curcumin explained many of its wide-ranging pharmacological activities. Curcumin was an effective scavenger of reactive oxygen and reactive nitrogen species in vitro (Joe and Lokesh, 1994) and inhibited inducible nitric oxide synthase activity in macrophages that were involved in inflammation and carcinogenesis (Brouet and Ohshima, 1995). The phenolic, methoxy groups on the benzene rings and the 1,3-diketone system are believed to be the two important structural features that contributed to its anti-oxidant properties (Sreejayan and Rao, 1996, 1997). Masuda et al. (2001) studied the oxidative coupling products of curcumin with a polyunsaturated fatty acid, linoleate and concluded that its anti-oxidant property was due to its chain-breaking reaction at the 3’-position with the lipid.

Other studies that indicated curcumin’s anti-oxidant activities, include the report by Reddy and Lokesh (1996) that oral administration of 30 mg curcumin/kg bw in rats for 10 days reduced the iron-induced hepatic damage by lowering lipid peroxidation. Lipid peroxidation was also lowered in liver, kidney, spleen and brain microsomes from retinol deficient rats that were fed 0.1% dietary curcumin for three weeks (Kaul and Krishnakantha, 1997). Another mechanism by which curcumin might protect against oxidative stress in endothelial cells was by the induction of heme oxygenase-1 (Motterlini et al., 2000). Curcumin pre-treatment decreased ischemia-induced changes in the feline heart (Dikshit et al., 1995) and also protected renal and neural glial cells
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from oxidative stress (Cohly et al., 1998). Dietary curcumin also induced protection from radiation in mice.

Interestingly, curcumin not only exhibited anti-oxidative and free radical scavenging properties, but also enhanced the activities of other anti-oxidants, such as superoxide dismutase, catalase and glutathione peroxidase (Reddy and Lokesh, 1994). It may also function indirectly as an anti-oxidant by inhibiting the activity of inflammatory enzymes or by enhancing the transcription of the genes for glutamate cysteine ligase (GCL), the rate-limiting enzyme in glutathione synthesis (Dickinson et al., 2003, 2004).

Due to its limited bio-availability in humans through the oral route, curcumin concentrations in plasma and tissue were likely to be much lower than those of other fat-soluble anti-oxidants, such as alpha-tocopherol (vitamin E). However, it has been reported that oral curcumin supplementation (3.6 g/day) for 7 days decreased the number of oxidative DNA adducts in malignant colorectal tissue, suggesting that curcumin taken orally may reach sufficient concentrations in the gastrointestinal tract to inhibit oxidative DNA damage (Garcea et al., 2005).

2. Anti-inflammatory Activity

Inflammation results from a series of complex reactions, triggered by inflammatory mediators, some of which were modulated by curcumin (Srimal and Dhawan, 1973). Membrane phospholipids are hydrolyzed by phospholipase A2 (PLA2), releasing arachidonic acid, which may be metabolized by cyclooxygenases (COX) to form prostaglandins and thromboxanes, or lipoxygenases (LOX) to form leukotrienes. Nuclear factor-kappa \( \beta \) (NF-\( \kappa \)\( \beta \)) is a transcription factor that binds DNA and enhances the transcription of the COX-2 gene and other pro-inflammatory genes, such as iNOS. In inflammatory cells, such as macrophages, iNOS catalyzes the synthesis of nitric oxide, which can react with superoxide to form peroxynitrite, a reactive nitrogen species that can damage proteins and DNA.

Curcumin inhibited the catalytic activity of 5-LOX directly, PLA2 by preventing its phosphorylation and COX-2 by inhibiting its transcription in cultured cells (Hong et al., 2004). It also inhibited NF-\( \kappa \)\( \beta \)-dependent gene transcription (Plummer et al., 1999) and inducible nitric oxide synthase (iNOS) in cell culture and animal studies (Brouet and Ohshima, 1995; Nanji et al., 2003).

Oral administration of curcumin was found to be effective in acute and chronic
inflammation (Arora et al., 1971; Mukhopadhyay et al., 1982) and in monkeys, it was shown to inhibit neutrophil aggregation associated with inflammation (Srivastava, 1989). Curcumin may also be applied topically to animal skin to counteract inflammation and irritation associated with inflammatory skin conditions and allergies (Mukhopadhyay et al., 1982).

3. Effects on Bio-transformation Enzymes Involved in Carcinogen Metabolism

Curcumin inhibited increase in cytochrome P450 1A1 (CYP1A1) activity, which is reported to be involved in the metabolic activation of several chemical carcinogens in cell culture and animal studies (Ciolo et al., 1998; Singh et al., 1998; Thapliyal and Maru, 2001; Rinaldi et al., 2002). However, increase in bio-transformation enzyme activity may enhance the elimination of potential carcinogens, but some carcinogen precursors (procarcinogens) were metabolized to active carcinogens by phase I enzymes (Baird et al., 2005). Several studies in animals have found that dietary curcumin increased the activity of phase II enzymes, such as glutathione S-transferases (GSTs) (Susan and Rao, 1992; Singh et al., 1998; Iqbal et al., 2003).

Dinkova-Kostova and Talalay (1999) studied a series of compounds structurally related to curcumin and concluded that the presence of two structural elements: (1) hydroxyl groups at ortho-position on the aromatic rings and (2) \( \beta \)-diketone functionality, were required for high inducer potency of phase II detoxifying enzymes. Curcumin has been shown to modulate glutathione (GSH)-linked detoxification mechanisms in vitro in human leukemia cell (K562 cells) and in vivo in rats (Piper et al., 1998). When rats were fed curcumin at doses from 1-500 mg/kg bw daily for 14 days, induction of hepatic GST activity increased in a dose-dependent manner (Piper et al., 1998). Curcumin treatment also caused a significant induction of an isozyme of glutathione S-transferase (GST) in rat lens epithelium, a property that could be useful for the management of cataractogenesis induced by lipid peroxidation (Awasthi et al., 1996). On the other hand, curcumin intake ranging from 0.45-3.6 g/day for up to 4 months was not shown to increase leukocyte GST activity in humans (Sharma et al., 2004).

4. Induction of Cell Cycle Arrest and Apoptosis

Curcumin is an apoptotic agent that induced cell cycle arrest and apoptosis in a number of cancer cell lines (Kuo et al., 1996; Duvoix et al., 2005; Sharma et al., 2004; Awasthi et al., 1996).
Curcumin-treated cells exhibited typical features of apoptotic cell death, such as shrinkage, transient phosphatidylserine exposure, increased membrane permeability and decreased mitochondrial membrane potential. Low concentration of curcumin arrested cell proliferation in the G0-G1/G2/S phase, while its high concentration induced apoptosis in rat culture cells (Hanif et al., 1997; Chen and Huang, 1998; Chen et al., 1999).

Apoptosis induced by curcumin in human basal cells was dependent on a p53-signaling pathway (Jee et al., 1998). Curcumin accumulated in the plasma membrane, endoplasmic reticulum and nuclear envelope, thereby, producing apoptosis-like changes in plasma membranes in rat thymocytes (Jaruga et al., 1998). Ramachandran and You (1999) demonstrated apoptosis to be involved in the differential curcumin-induced inhibition of mammary epithelial and breast carcinoma cell growth. They suggested that genes associated with cell proliferation and apoptosis might be playing a role in the chemopreventive action of curcumin.

5. Anti-carcinogenic Effects-Inhibition of Tumour Invasion and Angiogenesis

Animal studies involving rats and mice as well as in vitro studies utilizing human cell lines have demonstrated curcumin's ability to inhibit carcinogenesis at three stages: tumour promotion (Kawamori et al., 1999), angiogenesis (Thaloor et al., 1998) and tumour growth (Limtrakul et al., 1997). Curcumin has been found to inhibit the activity of several matrix metalloproteinases, which help cancerous cells to invade normal tissue in cell culture studies (Menon et al., 1999; Ohashi et al., 2003; Banerji et al., 2004). In two studies on colon and prostate cancers, curcumin was found to inhibit cell proliferation and tumour growth (Hanif et al., 1997; Dorai et al., 2001). It also inhibited angiogenesis (a process of developing new blood vessels) by invasive tumours in cultured vascular endothelial cells (Thaloor et al., 1998) and in an animal model (Arbiser et al., 1998). Further studies showed that curcumin prevented cancer in many tissues of mice and rats and has been associated with regression of established tumor malignancies in humans (Kuttan et al., 1987; Plummer et al., 2001).

The anti-carcinogenic effects of turmeric and curcumin were partly due to direct anti-oxidant and free radical scavenging effects. They also enhanced the body's natural anti-oxidant system, increasing glutathione levels, thereby aiding in hepatic detoxification of mutagens/carcinogens and inhibiting nitrosamine formation (Pizorrno and Murray, 1999).
The issue of concern is the observation that curcumin inhibited the cellular growth of both transformed and non-transformed cells in clonogenic assays, without discriminating between them (Gautam et al., 1998). However, this observation is yet to be confirmed and might have implications on the development of curcumin as an anti-cancer agent (Joe et al., 2004).

**Effects of Curcumin on Liver**

Turmeric has been found to have hepatoprotective characteristics. Curcumin administration significantly decreased certain liver enzymes, resulting in decreased liver injury following acute and sub-acute exposures to carbon tetrachloride in rats (Deshpande et al., 1998 a and b; Park et al., 2000). Curcumin’s hepatoprotective effect were also reported for galactosamine (Kiso et al., 1983), acetaminophen (paracetamol) (Donatus et al., 1990) and Aspergillus aflatoxin (Soni et al., 1992) and was mainly a result of turmeric's anti-oxidantive properties. Sodium curcuminate, a salt of curcumin, was shown to increase biliary excretion of bile salts, cholesterol and bilirubin, as well as increasing bile solubility, thereby possibly preventing and treating choledolithiasis (Ramprasad and Sirsi, 1957).

Deshpande et al. (1998a) reported decreased body weights and hepatotoxic effects expressed as focal necrosis, in rats and mice fed either turmeric or ethanolic turmeric extract, especially in high doses for prolonged periods. Similarly, Kandarkar et al. (1998) observed coagulative necrosis with areas of parenchymal regeneration following administration of whole spice turmeric or ethanolic turmeric extract for 14 days, at doses considered to be cancer protective. On the other hand, no toxic effects due to feeding of turmeric (or curcumin) were reported in rats, guinea pigs, monkeys and pigs (Wahlstrom and Blennow, 1978; Bhavanishankar et al., 1980; Bille et al., 1985). Al Sultan et al. (2004) reported hyperaemia of portal vessels and mononuclear cell infiltration in parenchyma and portal areas of broiler chicken that received dietary supplement of curcumin. But the region of most prominent histopathological changes was the bile duct where proliferation of bile duct epithelium and peri-portal hepatocyte degeneration was evident, possibly due to leakage of bile from the dilated bile ducts (Al Sultan et al., 2004).

**Effects on Renal System**

Babu and Srinivasan (1998) suggested that dietary curcumin ameliorated renal lesions
in streptozotocin-induced diabetic rats which was assessed in terms of the amount of proteins excreted in urine and the extent of leaching of renal tubular enzymes: N-acetyl glucosamine (NAG), lactate dehydrogenase (LDH), aspartate and alanine aminotransferase and alkaline and acid phosphatases. The integrity of kidney was assessed by measuring the activities of several key enzymes of renal tissue: glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, LDH, aldose reductase, sorbitol dehydrogenase, transaminases, ATPases and membrane polyunsaturated fatty acid (PUFA) / saturated fatty acid (SFA) ratio (membrane integrity). Cohely et al. (1998) reported decreased release of chromium-51 (an indicator of cellular damage to kidney epithelial cells) and increased $^3$H-arachidonic acid (an indicator of lipid degeneration) following curcumin and turmeric administration during H$_2$O$_2$-induced oxidative stress in renal epithelial cell line (LLC-PK1).

Curcumin protected against ADR induced renal injury by suppressing oxidative stress, increasing kidney glutathione content and glutathione peroxidase activity. Similarly, curcumin inhibited adriamycin stimulated kidney microsomal and mitochondrial lipid peroxidation (Venkatesan et al., 2000) and oxidative renal damage induced by ferric nitriloacetate in male ddY mice (Okada et al., 2001). The combination of mycophenolic acid with curcumin and quercetin was shown to reduce renal injury in rats (Jones and Shoskes, 2000). These data suggested that administration of curcumin is a promising approach in the treatment of renal disorders (Joe et al., 2004). Histopathological alterations in relation to ultrastructural changes in kidney post curcumin treatment have not been reported so far.

Other effects

Turmeric's protective effects on the cardiovascular system include lowering of cholesterol and triglyceride levels, decreasing susceptibility of low-density lipoprotein (LDL) to lipid peroxidation (Ramirez-Tortosa et al., 1999) and inhibiting platelet aggregation (Srivastava et al., 1986). Constituents of *Curcuma longa* exerted several protective effects on the gastrointestinal tract and were found to be capable of increasing gastrin, secretin, bicarbonate and pancreatic enzyme secretion (Ammon and Wahl, 1991). Turmeric extract and the essential oil of *Curcuma longa* have antimicrobial effects and inhibited the growth of a variety of bacteria, parasites and pathogenic fungi (Apisariyakul et al., 1995; Allen et al., 1998; Rasmussen et al., 2000).
Disease Prevention

The ability of curcumin to induce apoptosis in cultured cancer cells by several different mechanisms has generated scientific interest in its potential to prevent some types of cancer (Sharma et al., 2005). Oral curcumin administration has been found to inhibit the development of chemically induced cancers of oral cavity (Krishnaswamy et al., 1998; Li et al., 2002), stomach (Huang et al., 1994; Ikezaki et al., 2001), liver (Chuang 2000) and colon (Rao et al., 1995; Pereira et al., 1996; Kawamori et al., 1999) in animal models.

Curcumin or turmeric has also been recommended for preventing cataracts (Pandya et al., 2000; Awasthi et al., 1996), Alzheimer’s disease (Lim et al., 2001; Yang et al., 2005), muscle regeneration (Thaloor et al., 1999), accelerated wound healing (Sidhu et al., 1998, 1999) and treating high cholesterol (Ramirez-Tortosa et al., 1999), multiple sclerosis, fungal infections (Apisariyakul et al., 1995) and chronic anterior uveitis (Lal et al., 1999, 2000).

Safety and Dosage

No significant toxicity has been reported so far following either acute or chronic administration of turmeric extracts at lower doses and were generally limited to mild stomach distress. The pharmacological safety of curcumin was reported to be up to 100 mg/day and 5 g/day in humans and rats, respectively (Commandeur and Vermeulen, 1996). At very high doses, curcumin may be ulcerogenic in animals, as evidenced in a study on rats (Ammon and Wahl, 1991). Turmeric is on the FDA’s GRAS (generally recognized as safe) list (World Drug Information, 2006), and curcumin too, is believed to be fairly nontoxic (Ireson et al., 2001).

Genomic Analysis in Relation to Toxicity

An important advantage of genomic analysis during toxicological investigations is that gene expression changes are likely to be an initial response compared to more traditional toxicological endpoints. This allows increased sensitivity, earlier detection and measurement of toxicant effects at more environmentally relevant concentrations (Aardema and MacGregor, 2002; Waters and Fostel, 2004). Recent studies in rodents also suggested that genomic analysis may offer improved analysis of the effects of a complex mixture (Aardema and MacGregor, 2002; Amin et al., 2002).

One such DNA-based assay involves measurement of specific nucleotide changes...
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in genes of chemically exposed animals using restriction fragment length polymorphism (RFLP). Frequent mutations (interstitial deletion or missense mutations) in the \( \beta\)-catenin gene in rat, mouse and human liver neoplasms have been reported (De La Coste, et al., 1998; Miyoshi et al., 1998; Huang et al., 1999; Yamada et al., 1999; Ogawa et al., 1999). These findings suggested an important role of this gene in malignant transformation as observed previously in a variety of other tumours (Calvisi et al., 2001).

Catenins

The catenins are a multigene family of cytoplasmic proteins comprising \( \alpha\) (102 kDa), \( \beta\) (92 kDa), \( \gamma\) (Plakoglobin; 83 kDa) catenin and p120\(^{CAS}\) (Smith and Pignatelli, 1997). The catenins are multifunctional proteins that play a role in cell motility, growth, adhesion and signaling.

\( \beta\)-catenin is a multifunctional protein that acts both as a structural component of the cell adhesion machinery and as a transducer of extracellular signals (Tucker et al., 2003). It is a key regulator of the cadherin-mediated cell-cell adhesion system (Fig. 2.4) as it links the cytoplasmic domain of cadherins to \( \alpha\)-catenin, which anchors the adhesion complex to the cytoskeleton (Gumbiner, 1995).

\( \beta\)-catenin also plays a pivotal role in the Wnt-signal transduction pathway. Protein levels of \( \beta\)-catenin are regulated by phosphorylation at the NH\(_2\)-terminal region by GSK-3\( \beta\) complex that consists of adenomatous polyposis coli (APC) protein, axin/conductin and glycogen synthase kinase-3\( \beta\) (GSK-3\( \beta\)) (Fig. 2.5). Once phosphorylated, \( \beta\)-catenin was rapidly degraded through the ubiquitin-proteosome pathway (Munemitsu et al., 1996; Aberle et al., 1997). Mutations of APC or \( \beta\)-catenin itself (Polakis, 1999) lead to elevated free \( \beta\)-catenin, which translocated into the nucleus where it interacted with transcription factors of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family (Behrens et al., 1996; von de Watering et al., 1997). \( \beta\)-catenin/TCF complex activated target genes (He et al., 1998) such as c-myc, c-jun and c-fos (Abshire et al., 1996; Joseph et al., 2001; Spruill et al., 2002) that were
involved in the control of cell growth and apoptosis (Harris, 1991). The data strongly suggested that mutations enabling β-catenin to evade degradation by the proteosome complex play a fundamental role in the tumourigenic transformation of many cell types.

Mutations in β-catenin occurring in the four regulatory phosphorylation sites (codons 33, 37, 41 and 45) located at the N-terminus were causatively associated with a variety of human malignancies including tumours of skin, brain, kidney and colon (Koesters et al., 2001). They were found in ~50% of human colon tumours possessing an intact APC gene (Damalas et al., 1999; Koesters et al., 2001). A mutation in the GSK-3β consensus motif of β-catenin has been reported in rats and mice (Takahashi et al., 2000).

It may, thus, be concluded that β-catenin gene (CTNNB1 in humans and Ctnnb1 in rats/mice) activation and subsequent up-regulation of Wnt-signalling, was an important event in the development of certain human and rodent cancers (Devereux et
Delineating the Objectives of the Study

Large number of studies and a number of review articles on Cd toxicity are available. In most of these studies a single experimental parameter has been investigated. To the best of our knowledge, attention has not been paid to more than one parameter at a time and their mutual relationship. Even there is hardly any report on the comparison of toxic effects following acute and chronic Cd exposures. Hence, this study was conceptualized to identify in a rational and systematic manner Cd toxicity following acute and chronic exposures in mice liver and kidney, by assessing alterations occurring at the tissue, cellular and molecular level.

There has been a surge in efforts to identify natural compounds that may alleviate the risk of disease in recent times. Curcumin, a phenolic compound, is one such natural phytoneutritient, that possesses an array of bio-protective properties and has been reported to prevent onset/progression of serious diseases and limit the effects of the aging process. Most of the work on the protective role of curcumin deals with repeated exposures for longer durations with hardly any report on the effect of single exposure for short durations (24 hours).

Ebyl et al. (2004, 2006) reported the protective role of pre-treatment (3 days) with curcumin or its Mn-complex on Cd-induced oxidative damage and status of trace elements in tissues of rodents. Their studies involved biochemical measurements of LPO, GSH, GPx and catalase to assess oxidative damage. The related parameters of measurements of ROS generated and activities of other anti-oxidant and detoxifying enzymes have been skipped. Further, no morphological studies have been done. Likewise, the literature is scanty regarding the effect of curcumin and Cd administration on morphological alterations and genotoxicity.

Therefore, this work emphasized on the study of acute and chronic Cd toxicity and the modulating role of a single, short-term (24 hours) curcumin pre-treatment on acute Cd exposure in liver and kidney of mice. Hence, a multiparametric approach was used involving the investigation of the following parameters:

- Morphological alterations (i.e., histopathological and ultrastructural) were studied using light and scanning electron microscopes.
- Biochemical measurements of lipid peroxidative indices (lipid
peroxidation, hydroperoxide and superoxide anion generation), non-
enzymatic anti-oxidants (total cellular thiols and reduced glutathione), anti-
oxidant and detoxifying enzymes (superoxide dismutase, catalase, 
glutathione peroxidase, glutathione reductase and glutathione-S-
transferase), indicative of oxidative stress were measured 
spectrophotometrically.

- DNA laddering, a marker of DNA apoptosis, was observed using agarose 
gel electrophoresis.

- PCR-RFLP analysis of $\beta$-catenin gene was done to ascertain whether this 
gene was the possible target of Cd and curcumin genotoxicity. PCR 
primers for detection of $\beta$-catenin mutations were designed to amplify exon 
3 for the $\beta$-catenin gene containing consensus sequence for GSK-3$\beta$ 
(glycogen synthase kinase- 3$\beta$). The PCR products were treated with 
restriction enzymes Hinfl and EcoRI to detect mutations at codons 32, 33 
and 34 of the $\beta$-catenin gene.