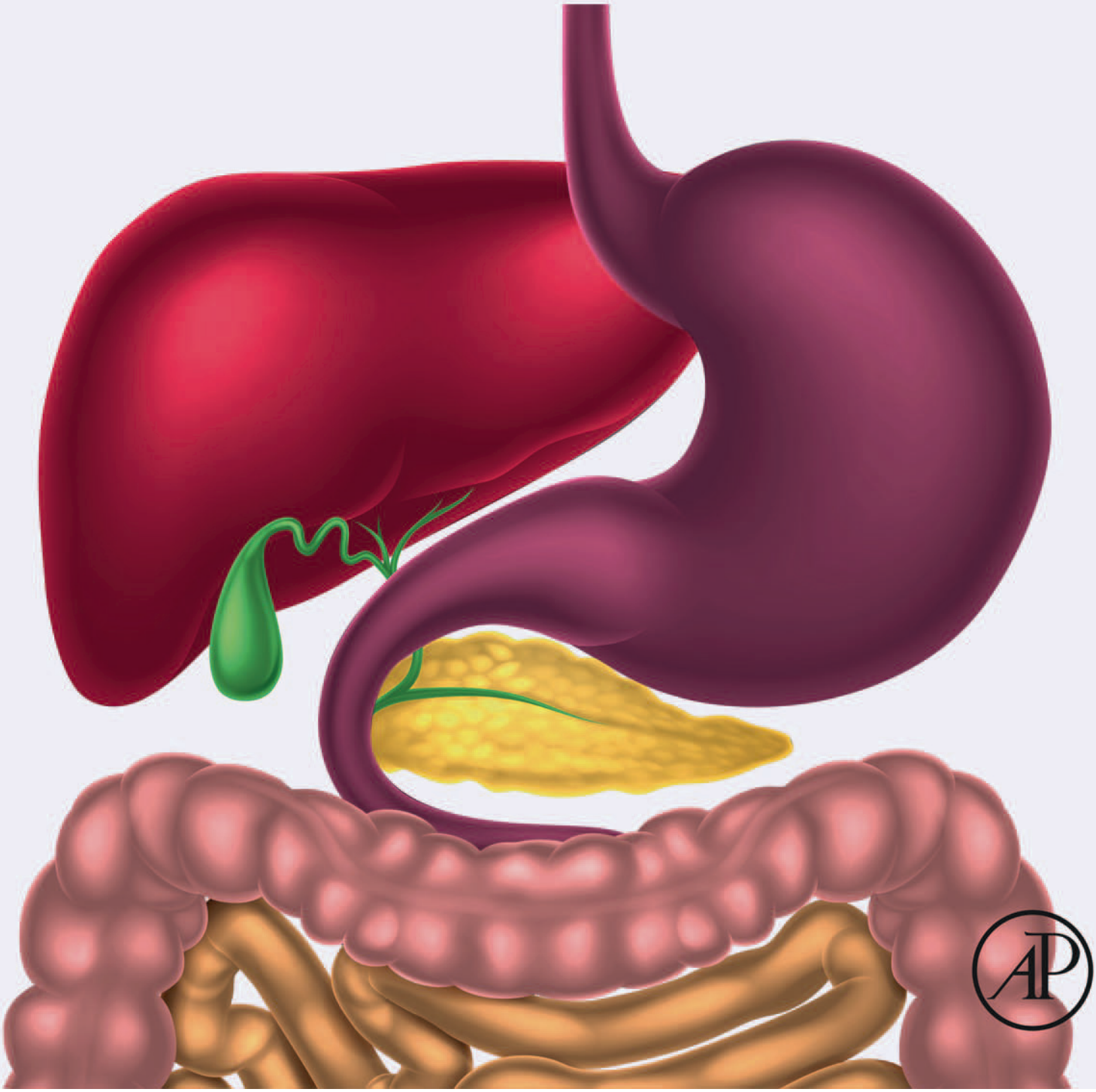
Dietary Interventions in Liver Disease

Foods, Nutrients, and Dietary Supplements

Edited by Ronald Ross Watson and Victor R. Preedy



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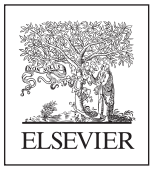

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Chapter 26

Heavy Metals and Low-Oxygen Microenvironment—Its Impact on Liver Metabolism and Dietary Supplementation

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1. INTRODUCTION

The **term heavy** metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. The heavy metals constitute major fraction of the periodic table and are generally interpreted to include those metals from periodic table groups IIA through VIA. Examples of heavy metals are nickel, lead, mercury, cadmium, chromium, etc. Heavy metals cannot be degraded or destroyed. It enters in our bodies via food, drinking water, and air and primarily targets liver and other metabolically active tissues. As trace elements, some heavy metals (e.g., copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning may occur through various ways such as drinking water through lead pipe or occupational exposure (lead–cadmium or nickel–cadmium batteries) or stainless steel industries (nickel-chromium), refineries or petrochemicals (nickel, lead, cadmium), jewelry, etc.[1] Hypoxia belongs to the most serious factors that can directly impair the function of metabolic pathways in the animal cell. The exposure of experimental animals to hypoxia has been widely used in many morphological and physiological studies. Physiological hypoxia induces cell signaling process for the formation of new blood vessels (angiogenesis) to regulate vascular tone during developmental stage.[2] Physiological oxygen levels (PO_2) in healthy body varies from −100 Torr in the alveoli to <10 Torr in medulla of kidney and retina.[3] Tissue exposure to low-oxygen tension is observed in several physiological and pathological conditions such as ischemia for shorter duration or in case of the high-altitude inhabitants or any other chronic diseases for longer duration of hypoxic exposure. In both cases, hypoxic cells are programmed to rapid adjustment to maintain O_2 supply to most vital organs such as heart and brain. It is understood that atherosclerosis, stroke, or vascular occlusion leads to tissue ischemia followed by hypoxia. Tissue hypoxia also develops through immune cell infiltration in vascular dysfunction during chronic inflammation process.[3,4] It has been observed that hypoxia absurdly stimulates free radicals release from the mitochondria that control the transcriptional and posttranslational response to low-oxygen conditions.[5] Hypoxia-induced generation of reactive oxygen species (ROS) has been a subject of theoretical and practical dispute as experimental designs able to quantitatively evaluate ROS formation. Under normoxic conditions, ROS (constantly generated in erythrocytes) are mostly counteracted by their endogenous (superoxide dismutase, glutathione peroxidase, catalase or reduced glutathione) or exogenous (vitamin C, vitamin E, etc.) antioxidant defense systems. Studies also show that wild-type human hepatoma cells (Hep3B) increase ROS generation of metal-activated cell signaling pathways during hypoxia.[5,6] Valko M et al.[7] stated that "hypoxia-activated gene transcription via a mitochondria-dependent signaling process induces increased ROS." The mechanisms by which mammalian cells adapt to acute and chronic alterations of oxygen tension are extremely important to understand the exact homeostasis regulation to counteract hypoxia-induced cell damage as a therapeutic strategy. Heavy metals are capable to induce expression of HIF-1 transcriptional factor and vascular endothelial growth factor (VEGF) genes through the

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phosphatidylinositol 3-kinase or Akt pathway or ROS.[8] Heavy metals–induced alteration of the hypoxia signaling system influenced by metal-induced oxidative stresses are responsible for progression of metastasis.[9] This chapter gives a brief understanding of current state of knowledge of chronic hypoxia and its influence on generation of ROS by inducing oxidative stress in the physiological system. The review will also provide recent update of heavy metal nickel toxicities on oxidant and antioxidant balance and molecular interaction of chronic hypoxia and heavy metal nickel (Ni) in the physiological system in vivo. Cellular hypoxia causes an initiation of hypoxia-response genes responsible for angiogenesis, oxygen transport, and metabolism.[10] Chronic hypoxia stimulates NF-κB gene expressions and it reduces KLF4, which further leads to an enhanced NOS2 expression (Fig. 26.1). **Both hypoxia and heavy metal exposure induce generation of ROS and increase expression of p53, NF-kβ, AP-1, MAPK, and HIF-1α. The increase expression of all these transcription factors leads to either cellular adaptation or cell death**.[11] **It is also to be mentioned that** hypoxic injury due to metal assault or hypoxia exposure causes "cell death" by cells swelling, plasma and nuclear membrane disruption, cellular lysis in association with acute inflammation that may exacerbate the initial hypoxic injury response. However, the alternative mode of cell death, apoptosis, is also possible (Fig. 26.1). During apoptosis, the cells use their molecular machinery to shrink or expand into membrane-bound apoptotic bodies, with or without nuclear fragments that are easily phagocytosed by adjacent tissue cells or macrophages and minimize any acute inflammatory response.

Liver is an important metabolically active organ. It stores additional nutrients in the form of glycogen and lipids. During the need of the hour these nutrients yield energy and keep all the vital functions intact. Hepatocytes also synthesize plenty of proteins including albumin and clotting factors. Furthermore, it synthesizes cholesterol and triglycerides. Another important function of liver is to produce bile salts which are essential for digestion and absorption of lipids. The hepatocytes also play an important role as the center of detoxification in the body, influencing drug metabolism and breakdown of hormones. This organ is an important source of storage of vitamins such as B12, A, D, K and folic acid, besides being an important source of iron. To make the liver a well-functioned organ, a considerable amount of oxygen is needed. Altered metabolic functions due to toxic insults or metabolic stress due to hypoxia or heavy metal toxicities disturb oxygen homeostasis in liver and lead to serious liver diseases. Most of the cases, malfunction of liver leads to fatty liver symptoms and the cell signaling pathways greatly affected is oxygen dependent, hence hypoxia may be considered as an important cause of liver malfunction.[12] Interestingly, hypoxia and divalent heavy metals such as nickel (Ni) and lead (Pb) generate ROS and disturbed oxidant/antioxidant balance which is linked to the transcriptional factor HIF-1α. The results from the author's laboratory showed both divalent cationic heavy metal (Ni and Pb) and chronic sustained hypoxia stimulate the production of HIF-1α transcription factor and VEGF gene expression in metabolically active tissues in similar molecular mechanism. Heavy metals cause oxidative stress by inducing the generation of ROS; reducing the antioxidant defense system of cells via depleting glutathione; interfering with some essential metal; inhibiting sulfhydryl (SH), dependent enzyme, or antioxidant enzymes activities; and/or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition.[13,14]

Nutrients such as vitamin C or E are found to be the most effective circulatory antioxidant in human system.[15] Ascorbic acid or vitamin C prevents lipid peroxidation, oxidation of low-density lipoproteins, and advanced oxidation protein products.[16] Vitamin C may comprise the first line of defense system in RTLF against external pro-oxidative assaults.[17] It has been reported that intracellular depletion of ascorbic acid aggravated some heavy metal (nickel, cobalt, etc.)-induced carcinogenicity and acute toxicity.[18] The effect of simultaneously supplemented vitamin C on experimental nickel treatment

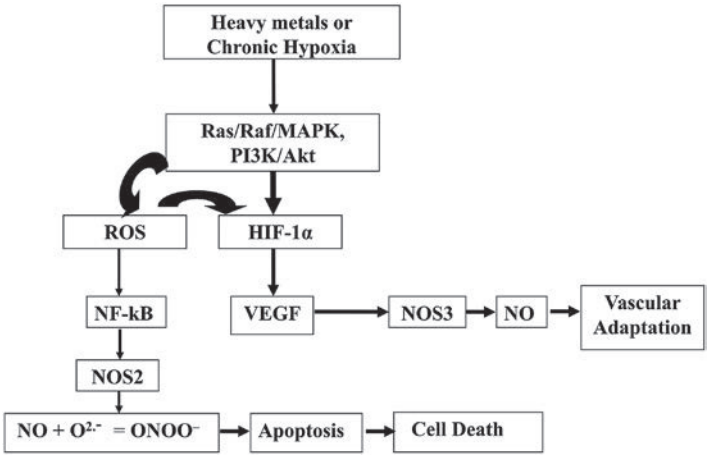

FIGURE 26.1 Graphic representation showing heavy metals or hypoxia-induced common cellular abnormalities. *NF- kB*, nuclear factor-kappa B; *NO*, nitric oxide; *NOS*, nitric oxide synthase; *ONOO⁻*, peroxynitrate; *HIF-1α*, hypoxia inducible factor 1α; *ROS*, reactive oxygen species; *VEGF*, vascular endothelial growth factor.

shows ascorbic acid is capable to reduce intestinal absorption of nickel. The mechanism involves that vitamin C is capable to reduce ferric iron to ferrous iron in the duodenum, thus availability of divalent ferrous ion increases which competes with nickel or lead also as divalent cation for intestinal absorption.[19] Recent reports indicate the capability of ascorbic acid as a regulatory factor may influence gene expression, apoptosis, and other cellular functions of living system exposed to heavy metals.[20] This chapter elaborately explains the role of dietary supplementation of nutrients such as vitamins and other nutrients in heavy metals such as nickel and lead, which induces altered hepatic functions in low-oxygen microenvironments.

2. HEAVY METALS AND ITS INTERACTIONS

Heavy metals interact with the biological system in a complex manner. Even elemental speciation of the metals also matters in its interactions with systems. The **term heavy** metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. The heavy metals constitute major fraction of the periodic table and are generally interpreted to include those metals from periodic table groups IIA through VIA. Examples of heavy metals are nickel, lead, mercury, cadmium, chromium, etc.[1]

2.1 Heavy Metal Toxicities: Nickel and Lead

There are five priority substances which are selected by WHO for the nickel risk assessment. They are nickel powder, nickel sulfate, nickel chloride, nickel carbonate, and nickel nitrate. Nickel powder (T; R48-23) has been classified in chronic toxicity classification as per environmental risk assessment report on nickel. $NiSO_4$, $NiCl_2$, $NiCO_3$, and $NiNO_3$ are classified as carcinogen class I (by inhalation), reproductive toxicants class II (may cause harm to unborn children), and chronic toxicants (T; R48-23). If particle size of nickel powder found to be less than 0.1 mm, it is classified as T; R52-53 (harmful to the aquatic environment).[21] Acute toxicity in humans, which results from absorption through the gastrointestinal tract or by inhalation through lungs, was first reported by Sunderman in 1954.[22] Further studies showed that a single dose oral LD50 in rats for the less-soluble nickel oxide and subsulfide was >3600 mg Ni/kg bwt, whereas the oral LD50 for the more soluble nickel sulfate and nickel acetate ranged from 39 to 141 mg Ni kg^{-1} bwt in rats and mice.[23] The metal is not only an allergen but also a potential immunomodulatory and immunotoxic agent in humans.[19] Weischer et al.[24] reported that oral administration of nickel as NiCl2 in male rats over a period of 28 days at concentration of 2.5, 5.0, and 10.0 µg/mL in drinking water (0.38, 0.75, or 1.5 mg/kg day) resulted in significant dose-dependent hyperglycemia, decrease in serum urea, and significant increase in urine urea. At 0.75 mg/kg doses, increased leukocyte count was also observed. It was noticed that exposure of dietary nickel sulfate hexahydrate (100, 1000, or 2500 ppm) to dogs for 2 years failed to produce significant signs of compound-related toxicity.[25] The toxicity of the different nickel compounds is related to its solubility, with soluble nickel sulfate being the most toxic and insoluble nickel oxide being the least toxic. The difference in the toxicity across compounds is probably due to the ability of water-soluble nickel compounds to cross the cell membrane and interact with cytoplasmic proteins.[21]

Lead poisoning can affect almost all parts of the body, but its effects are most pronounced on the central nervous system and kidneys. Lead can impair cognitive development, which can lead to learning disabilities and behavioral problems. Acute lead exposure can cause encephalopathy, severe abdominal pain, vomiting, diarrhea, coma, seizures, and, in some cases, death. Chronic exposure can cause weakness, prolonged abdominal pain, anemia, nausea, weight loss, fatigue, headache, and loss of cognitive function. Chronic, low-level lead exposure can be asymptomatic until kidney function starts to deteriorate.[11] Lead has no known physiologically relevant role in the body, and its harmful effects are myriad. Lead and other heavy metals create reactive radicals, which damage cell structures including DNA and cell membranes. Lead also interferes with DNA transcription enzymes that help in the synthesis of vitamin D, and enzymes that maintain the integrity of the cell membrane. Anemia may result when the cell membranes of red blood cells become more fragile as the result of damage to their membranes. Lead interferes with metabolism of bones and teeth and alters the permeability of blood vessels and collagen synthesis. Lead may also be harmful to the developing immune system, causing production of excessive inflammatory proteins; this mechanism may mean that lead exposure is a risk factor for asthma in children. Lead exposure has also been associated with a decrease in activity of immune cells such as polymorphonuclear leukocytes. Lead also interferes with the normal metabolism of calcium in cells and causes it to build up within them. It is metabolized by CYP450 to trimethyl lead (TML). Mechanisms of its toxicity include damage to membranes, disturbances in energy metabolism, and direct interference with neurotransmitter synthesis. Symptoms of its toxicity include nausea, vomiting, diarrhea associated with nervous system problems such as irritability, headache, and restlessness. Chronic heavy sniffing of leaded gasoline results in signs of dementia and encephalopathy, with cerebellar and corticospinal symptoms. Lead primarily acts by competing with endogenous cations on protein-binding sites. In particular, lead can substitute both calcium and zinc in

numerous proteins. Among stress-response genes that were upregulated by lead treatment, GFAP, microsomal glutathione S-transferase, mitochondrial 10 KDa heat shock protein, and HSP70 are all involved in general cellular responses to stress. Daphnia hemoglobin gene was greatly expressed following lead exposure.[26]

3. HYPOXIA PATHOPHYSIOLOGY

Hypoxia is a pathological condition in which the body as a whole (generalized hypoxia) or a region of the body (tissue hypoxia) is deprived of adequate oxygen supply. Variations in arterial oxygen concentrations can be part of the normal physiology, for example, during strenuous physical exercise. In healthy humans, there is a range of physiological oxygen levels within the tissues of the body, ranging from PO2 values of −100 Torr in the alveoli of the lungs to less than 10 Torr in tissues such as the medulla of the kidney and the retina.[27]

3.1 Hypoxia Microenvironment

Physiological hypoxia is an important microenvironmental signal in a range of processes including new blood vessel formation (angiogenesis) during development and wound healing, the regulation of vascular tone, and the response to exercise. However, tissue hypoxia is also associated with a diverse and wide range of pathophysiological processes including (but not limited to) vascular disease, chronic inflammation, and cancer.[2] In vascular diseases such as atherosclerosis and stroke, vascular occlusion leads to acute or chronic tissue ischemia with resultant hypoxia. In chronic inflammatory diseases, the greatly increased metabolism of inflamed tissue due to immune cell infiltration matched with vascular dysfunction leads to tissue hypoxia.[27] Hypoxia results from conditions such as ischemia, hemorrhage, stroke, premature birth, and other cardiovascular difficulties. Among which hemorrhagic shock is the leading cause of death and complications in combat casualties and civilian settings. It has been shown to cause systemic inflammation response syndrome, multiple organ dysfunctions, and multiple organ failure.[28] Hypoxia has been shown to lead to increases in intracellular free calcium concentration (Ca^{2+}), 5-lipoxygenase, lipid peroxidation, cycloxygenase (COX), constitutive nitric oxide synthase (cNOS), leukotriene B4 (LTB4), prostaglandin E2 (PGE2), interlukins, tumor necrosis factor-α (TNF-α), caspases, complement activation, kruppel-like factor 6 (KLF6), inducible nitric oxide synthase (iNOS), heat shock protein 70 kDa (HSP-70), and hypoxia-inducible factor-1α (HIF-1α). The sequence of their occurrence provides the useful information for studying the mechanisms underlying the hypoxia-induced injury as well as therapeutic targets to prevent or ameliorate the injury.[11] Hypoxia, or inadequate oxygenation, causes various responses within the body. Its effects are usually mediated via the activation of HIF-1. HIF-1 activation can lead to upregulation of various genes such as erythropoietin and growth factors that help tissues adjust to the decreasing oxygen availability. Semenza and Wang defined a binding site critical for the hypoxia-inducible function, which involves a transcription factor induced by hypoxia. Subsequently, they purified a DNA-binding complex bound to the HRE by affinity purification using oligonucleotide with the HRE sequence and thus identified the encoding cDNAs.[29]

3.2 Hypoxia and Heavy Metals (Nickel and Lead)

Over the recent years, induction of signaling pathways that regulate key cellular responses related to cancer growth and progression by metals has been the focus of many studies. The unraveling of these pathways and the deciphering of their interplay with metals should allow a better understanding of metal toxicity and hopefully will enable development of prophylactic strategies and therapeutic approaches. Authors' laboratory and works of Leonard (2004) have shown the mechanisms of toxicities caused by heavy metals such as nickel and lead, emphasizing on the involvement of the hypoxia signaling pathway by metal-induced generation of ROS and oxidative stress generation.[20,30] Hypoxia-induced factor HIF-1 controls precise oxygen homeostasis by modulating expression of several cancer-related genes, including heme oxygenase 1 and vascular endothelial growth factor. The carcinogenic metals such as nickel, lead (Pb), or chromium have been known to activate HIF-1.[8,31] It has been observed that heavy metal–induced ROS generation during the exposure of cells to metals mimic hypoxia-like symptoms.[32] The mechanisms of carcinogenesis caused by heavy metals such as nickel emphasizes on the involvement of the hypoxia signaling pathway by metal-induced generation of ROS and oxidative stress generation in cancer progression.[9] One of the pathways by which heavy metals such as nickel and lead induce intracellular hypoxia is by reducing heme biosynthesis. Low level of heme reduces intracellular oxygen tension and simply intracellular low Fe^{2+} and low oxygen tension inhibit PHD_2 (prolyl hydroxylases). Under normoxic conditions, HIF-1-prolyl hydroxylases (PHD) hydroxylate the prolyl residues at amino acids 402 and 564. These enzymes require dioxygen, Fe^{2+}, ascorbate, and two oxoglutarates for activity. The hydroxylated peptides interact with an E3 ubiquitin-protein ligase complex composed of

pVHL (von Hippel–Lindau tumor suppressor protein), elongin B and C, and Cullin 2 (CUL2), and then poly-ubiquitinized, resulting in HIF-1α degradation by the 26S proteasome. Under hypoxic conditions, HIF-1α is not hydroxylated because the major substrate, dioxygen, is not available. The unmodified protein escapes the VHL-binding, ubiquitination, and degradation, and then dimerizes HIF-1α and stimulates the transcription of its target genes.[33]

4. HEAVY METALS IN LIVER DISEASES

Heavy metals related to cardiovascular and pulmonary disorders are quite common and reported elsewhere, but currently, heavy metals and its impact on liver disease are considered as serious as before.[34]

4.1 Heavy Metals and Liver Pathophysiology (Nickel and Lead)

Fatty liver disease is considered as one of the important causes of chronic liver disease, and it is manifested by a complicated etiology. Heavy metal–induced changes in liver pathophysiology including fatty liver changes are under non–alcoholic fatty liver disease (NAFLD) category. Fatty liver induces a prolonged inflammatory response which leads to fat accumulation in the liver due to hepatocellular damages. One study showed that heavy metals caused NAFLD in men under 24 BMI. In case of overweight and obese, it becomes more serious. It was also observed that lead (Pb) causes more liver damage than nickel (Ni).[35] Heavy metals such as nickel and lead cause hepatocellular hyperplasia, which may lead to even carcinoma of liver. Studies on nickel clearly showed elevation of liver aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltranspeptidase.[6,36] Furthermore, it has been found that at advanced stages of hepatic cirrhosis, there was a significant increase of hepatic levels of nickel.[37] Another study also showed lower serum nickel concentration in liver cirrhosis patients which attributes a possible reduction of hepatic synthesis of nickel transport protein, i.e., nickeloplasmin and albumin.[38] Similarly, study also showed that lead and mercury are linked with NAFLD.[39] Furthermore, it was observed that lead (Pb) become conjugated in liver and stored there in highest concentration. Lead exposure on experimental animal showed an elevation of AST, ALT, and alkaline phsophatase, which clearly indicate a possible liver failure.[40] The most common pathways for metal-induced hepatotoxicity is through free radicals due to oxidative stress. The free radicals which are generated due to heavy metal exposure damage cell membrane lipid bilayers, nucleic acids, and enzymes. These in turn causes functional impairment of cell integrity and disturbs cytoprotective systems. Furthermore, it imparts oxidant and antioxidant imbalances and leads to cellular injuries. The mechanisms of hepatotoxicities are through affecting hepatic mitochondrial respiratory systems by reducing cytochrome *c* oxidase activity. Excessive accumulation of heavy metals also disturb hepatic calcium regulatory system by damaging microsomal calcium sequestration and damaging hepatocellular DNA, which further leads to carcinoma of liver.[41]

4.1.1 Nickel and Hepatotoxicities

A transient increase in serum bilirubin was observed in 3 out of 10 workers who were hospitalized after drinking water from a water fountain, contaminated with nickel sulfate.[41] In rats, decreased liver weight was observed following exposure for 28 days to 2 year to 0.97–75 mg/kg day of nickel chloride or nickel sulfate.[42] Recent studies on rats by Das et al.[43] revealed a nickel sulfate–induced degenerative effect on hepatic tissue They have observed that after the intraperitoneal injection of nickel sulfate, normal hepatic architecture was greatly altered, along with appearance of vacuolated cytoplasm (fatty liver), eccentric nuclei, and Kupffer cell hypertrophy. One report described decreased hepatic and renal transaminase activities after nickel treatment in rats, which was found more deleterious in a protein-restricted dietary regimen.[44] Nickel sulfate also decreases the liver ascorbic acid and cholesterol levels in rats.[6] Misra and coworkers showed that a single intraperitoneal injection of nickel (II) acetate increased lipid peroxidation and glutathione-S-transferase activity in rat liver and kidney while concomitantly decreasing the glutathione concentration and glutathione reductase activity.[45] The same group found that the nickel-induced hepatic lipid peroxidation in different strains of mice was concurrent with nickel's effect on antioxidant defense systems in liver and kidney.[46] The magnitude of nickel-induced lipid peroxidation showed a reverse correlation with the extent and direction of its effect on glutathione and glutathione peroxidase glutathione reductase but not on CAT, SOD, or glutathione-S-transferase.[46] Das et al.[47] showed, after the nickel treatment of rats, a significant rise in hepatic lipid peroxides and a decrease in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities and in the hepatic glutathione concentration. The alteration of oxidant and antioxidant balance due to hepatic lipid peroxidation indicates an elevation of enzyme phospholipase activities while peroxidic disintegration of various subcellular organelles and membrane lipid layers with nickel exposure. Furthermore, it may be postulated that nickel causes Kupffer cell hyperactivity through inflammatory cytotoxic mediators along with fatty liver changes and

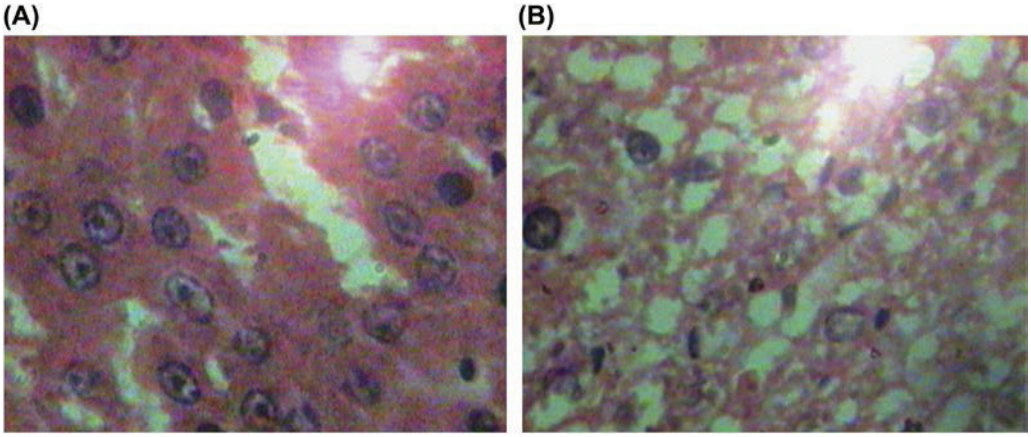

FIGURE 26.2 Normal (A) and nickel sulfate (B)–treated rat liver histopathology (45×).

eccentric nuclei in hepatocellular architecture. Nickel-induced changes of hepatic SOD, CAT, and GSH-Px reveal possible interaction of free radicals and hepatic enzymes and damaging SH protective mechanism against lipid peroxidation.[47] Study also revealed that nickel or some other heavy metals cause alteration in hepatic HMG-CoA reductase activities and disturb LDL-receptor gene expression. It ultimately changes the lipid profile of physiological system.[48,49] Another study showed that nickel induced severe liver damage as indicated by rise of SGOT, SGPT, and ascorbate-cholesterol metabolism in experimental rats. The study also showed that nickel sulfate causes decrease in absolute liver weight without altering hepatosomatic index which is indicative of hepatic degenerative changes.[50] Increased activity of SGOT and SGOT after nickel exposure reflects possible leakage of hepatic enzymes from liver cytosol in circulatory system due to nickel-induced cellular damages.[51] Another observation on nickel-induced hyperglycemia in experimental animals indicates a marked reduction in hepatic fructose-2-6-bisphosphate, which is an indicator of gluconeogenic and glycolytic pathways suggestive of increase of liver gluconeogenesis.[52]

In histopathological studies in the author's laboratory, the liver showed congestion of central veins and sinusoids and some hepatocytes suffered from vacuolar degeneration, fatty changes, etc. (Fig. 26.2A and B). Mathur et al. also observed the same in nickel sulfate–treated rats.[53] Results from the author's laboratory are in agreement with those obtained by El-Saeed and Mekawy[54], Ptashynski and Klaverkamp,[55] and Sobecka[56]. Nickel intoxication causes a vacuolization of the cytoplasm, the increase in numbers of pyknotic nuclei, and the decrease in glycogen content in hepatocytes.[57] The hydropic degeneration of hepatocytes may be due to the irritation of toxic metabolites and impairment of potassium sodium pump that disturbs the ion exchange through the cell wall. The increased oxidative stress, the formation of ROS as well as depletion of cellular antioxidant level may be resulted in histopathological changes of liver. Heavy metal–induced interstitial fibrosis, increased numbers of pyknotic nuclei, as well as necrosis in hepatocytes have also been reported earlier.[58]

4.1.2 Lead and Hepatotoxicities

Like nickel, lead too raises serum LDL-cholesterol, VLDL-cholesterol, total cholesterol and triglycerides, and decreases serum HDL-cholesterol and HDL/LDL ratio. It may be due to changes of the gene expression of hepatic enzymes and LDL receptor synthesis. Defects in the LDL-receptor interfere with cholesterol uptake from the bloodstream, which in turn causes excess cholesterol synthesis in the liver and high levels of serum total cholesterol and LDL-cholesterol.[48] The improvement of serum lipid profile also reflects normalization of liver P450 enzyme system function by α-tocopherol.[59] Lead generates long-lived ROS. These might cause oxidative stress that results in oxidative deterioration of biological macromolecules leading to oxidative damage to the hepatic cells.[60] In an experimental study, lead acetate induced increase plasma MDA with decreased hepatic SOD, CAR, and GSH-Px were noticed which are indicative of hepatic oxidative stress.[61] During hepatotoxicity, these enzymes are structurally and functionally impaired by free radicals, resulting in liver damage. Glutathione comprises up to 90% of the nonprotein thiol content of mammalian cells and performs a pivotal role in maintaining their metabolic and transport functions. It acts as a nucleophilitic "scavenger" of many compounds and their metabolites via enzymatic and chemical mechanisms, converting electrophilic centers to ether bonds. Glutathione depletion to about 20%–30% of total glutathione levels can impair cell defenses against toxic actions, which may lead to cell injury

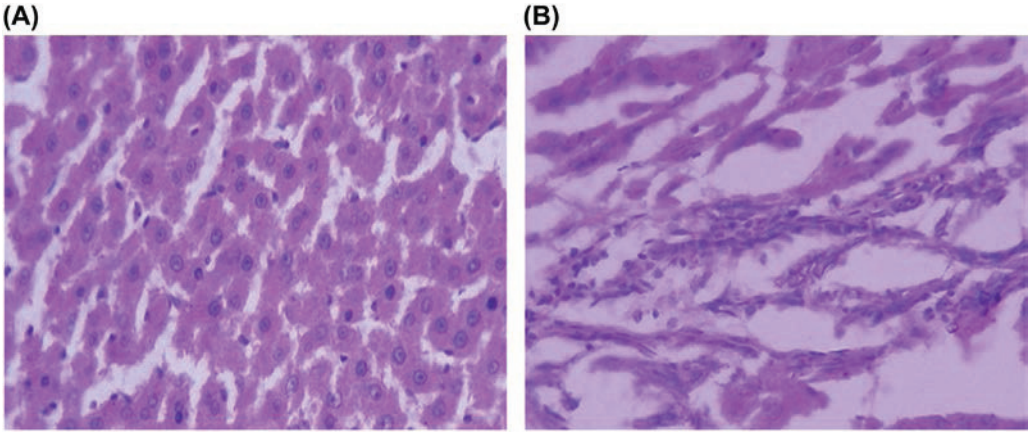

FIGURE 26.3 Normal (A) and lead acetate– (B) treated rat liver histopathology (45×).

and death.[21] Furthermore, glutathione is considered a crucial factor in maintaining the structural integrity of cell membranes, largely through reactions that protect the membrane against free radical formation.[62] Lead interacts with negatively charged phospholipids in membranes and through the induction of changes in membrane physical properties could facilitate the propagation of lipid oxidation in liver. Lead affects membrane-related processes such as the activity of membrane enzymes, endo- and exocytosis, transport of solutes across the bilayer, and signal transduction processes in hepatocytes by causing lateral phase separation.[30,61] Lead-induced oxidative stress in liver caused increase of rate of production of hydroxyl radicals which may lead to lysosomal and mitochondrial damages. Besides these direct hepatocellular damaging by lead-induced ROS and reactive nitrogen species (RNS), it may also interfere cell signal transduction by reversible oxidation and nitrosation of protein SHs in the hepatic sinusoid.[63]

Histopathological studies of lead-treated rat liver from the author's laboratory indicated little swollen hepatocytes with ill-defined cell borders with variation in cellular size and shape. The nuclei are large, more vesicular with variable size and shape, and contain multiple three to four prominent nucleoli. The cytoplasm is vacuolated and microvesicular. There are foci of fatty change and ballooning degeneration and necrosis of hepatocytes in zone 3 (centrilobular) areas.

The portal area appears mildly enlarged with mild proliferation fibrous tissue with infiltration of mixed acute and chronic inflammatory cells. The sinusoidal spaces are variably widened with increase in number of Kupffer cells. Central vein shows features of dilatation and congestion (Fig. 26.3B). Results clearly indicate hepatocellular damage by lead exposure.

4.2 Possible Mechanism of Altered Hepatocellular Architecture by Heavy Metals

It was found that most of the divalent heavy metals such as nickel, lead, and cadmium enter into systemic circulation from intestine through metal transporter proteins (MTP 1). Through circulation these metals enter first to liver via portal circulation where it is absorbed through sinusoidal capillaries. In hepatocytes, these heavy metals are penetrated through specific membrane transporters such as DMT1, ZIP8, and ZIP14t.[64,65]

Heavy metals such as nickel or lead accumulate in liver and resulting hepatocellular damages induce infiltration of polymorphonuclear neutrophils. This in turn causes activation of Kupffer cells followed by necrosis. Usually heavy metal–activated Kupffer cells secretes several inflammatory cytokines and causes secondary liver damage.[66] The exact mechanism of hepatocellular damages by Kupffer cells is yet to be cleared, but a possible role of free radicals, nitric oxide, tumor necrosis factor α (TNF-α) cannot be ruled out[67] (Fig. 26.4). Studies on nickel showed hepatic apoptosis due to overexpressions of caspase-3, caspase-9, and PARP mRNA.[68]

5. HYPOXIA AND LIVER DISEASES

Liver pathophysiology is oxygen dependent. As it is an important organ for metabolism, it is always in demand for oxygen. Hepatic artery, portal veins, and central veins play the pivotal role to maintain liver oxygen homeostasis.[69] It was found that liver always make oxygen microenvironment differentially than other organs. The important change

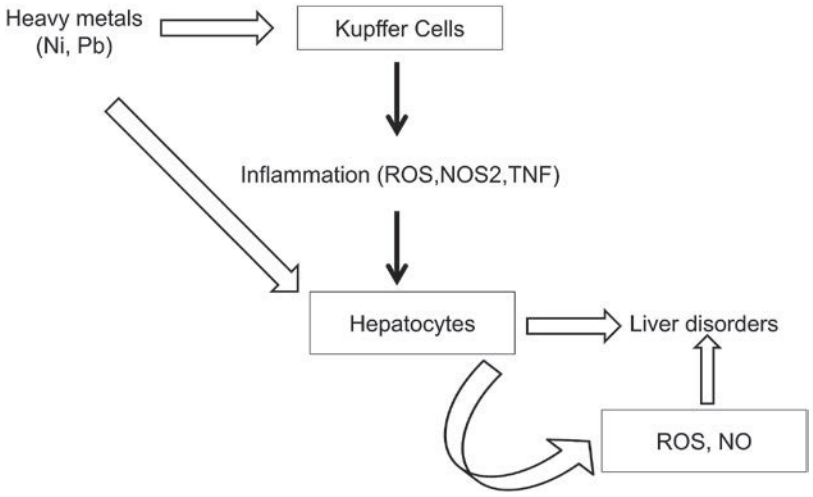

FIGURE 26.4 Heavy metal–induced liver malfunctions.

in hypoxia-induced hepatocellular architecture is the formation of plasma membrane protrusion. This formation has numerous cytosol and endoplasmic reticulum. It ultimately causes swelling of mitochondria and near 30%–50% increase of cell volume. These changes can be reversed if reoxygenation to hepatocytes occurs. The hepatocytes may be permanently injured if hypoxia sustains, and it will lead to complete damage of plasma membrane transport system which will cause release of intracellular ingredients of hepatocytes.[70] Oxygen tension in periportal and perivenous part of liver is 60–75 mmHg and 30–35 mmHg, respectively, which clearly indicates a persistent hypoxia in liver due to high metabolic functions.[69] Study reveals that hypoxia is linked to several types of liver diseases. The mechanism by which hypoxia is able to change liver pathophysiology is mainly through HIF-1 and NOS2 expressions. Both these factors are involved in hepatocytes, Kupffer cells, and immune cells. Hypoxia in liver increases the level of TNF-α, IL-1 from hepatocytes which further promote ROS. These ROS in liver are found to have decreased glutathione levels and elevated oxidized glutathione.[71] Although a direct hypoxia response to liver was not found in healthy individuals, in the case of viral hepatitis, metabolic diseases, steatohepatitis, and cancer, an elevation of HIFs is noticed. It has been observed that HIFs induce pathogenesis of hepatocellular carcinoma, and both HIF-1 α and VEGF levels were increased in hepatocellular carcinoma.[72] Many chronic liver diseases due to vital infection, metabolic disorders, or alcoholism are found to be connected with HIFs.[73] Actually HIFs act as protective agents from liver injuries due to hypoxia. HIFs induce generation of VEGF, adenosine, nitric oxide, and Akt signaling pathways to prevent hepatocellular injuries from hypoxia.[74,75] It is observed that Dec1 expression increases in alcoholic liver which indicates HIF-1α regulatory gene involvements to protect liver of alcohol toxicities. Hypoxia region of liver shows alteration of parenchymal vasculature, which leads to fibrosis.[76] HIF-1α expression stimulates hepatic stellate cells (HSCs) and fibroblasts. Another study on NAFLD phenotype showed hypoxia accelerated the NAFLD phenotype with higher level of lipogenesis and inflammation.[77] Another important phenomenon of hypoxia-induced liver injury is through ATP depletion during hepatic ischemia which also leads to necrotic cell death. Hypoxic liver enhances glycolytic metabolism and prevents its best against hypoxia injuries. In case of low glycogen in liver, hypoxia leads to rapid cellular ATP depletion and necrosis.[78] Hypoxia-exposed liver also shows alteration of pH microenvironment. Hypoxia leads to acidosis in liver, which prevents necrotic cell death in liver in spite of low ATP levels.[79]

5.1 Hypoxia—Liver Histopathology

In histopathological studies in the author's laboratory, the subchronic hypoxia–exposed rat liver showed endothelial cells surrounded by a ring of collagen fibers in the central vein. The sinusoids are lined by both endothelial cells and Kupffer cells both of which have inconspicuous flattened nuclei and ill-defined cytoplasmic margins. The hepatocytes are polygonal in shape with well-defined borders and appear to be little swollen with mild narrowing of the sinusoidal spaces. The nucleus is single, is round, and has a fine chromatin pattern with one to two clearly defined amphophilic prominent nucleoli. More or less it reflects normal architecture with insignificant changes in hypoxia-exposed rat liver (Fig. 26.5B).

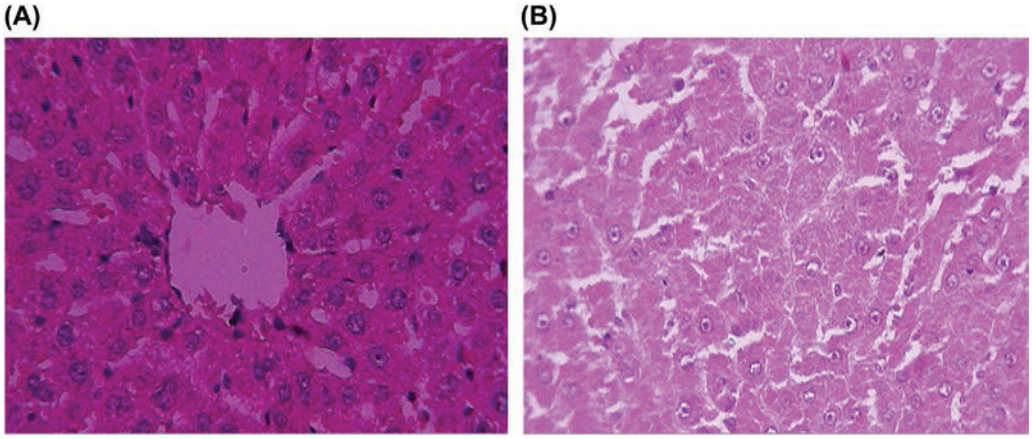

FIGURE 26.5 Normal (A) and chronic hypoxia- (B) exposed rat liver histopathology (45×).

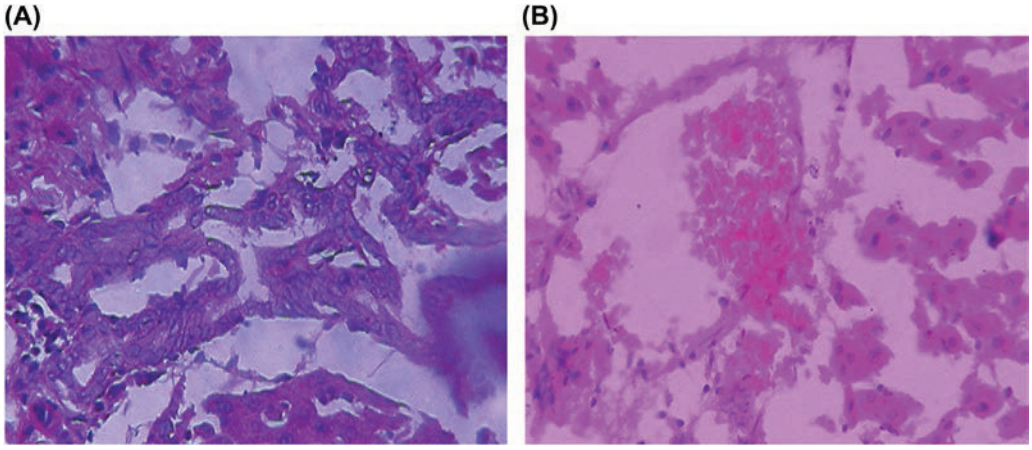

FIGURE 26.6 Hypoxia exposed with nickel sulfate– (A) and lead acetate– (B) treated rat liver histopathology (45×).

5.2 Hypoxia and Heavy Metals (Nickel and Lead)—Liver Histopathology

There is evidence of fatty change and ballooning degeneration and necrosis of hepatocytes. The portal area appears enlarged with severe proliferation fibrous tissue with infiltration of mixed acute and chronic inflammatory cells in nickel sulfate–treated subchronic hypoxia-exposed rats (Fig. 26.6A). In the case of subchronic hypoxia–exposed lead acetate–treated rats, distorted "lobular" architecture of liver parenchyma is noticed. Hepatocytes appear to be little swollen and cytoplasm is vacuolated, microvesicular, and eosinophilic (Fig. 26.6B). It also shows increase in number of mitotic figures along with foci of fatty change and ballooning degeneration and necrosis of hepatocytes in zone 3 (centrilobular) areas. Moderate proliferation of a portal area with fibrous tissue with infiltration of mixed acute and chronic inflammatory cells and variable widening of sinusoidal spaces along with Kupffer cell hyperplasia, dilatation, and congestion of central vein are also observed.

6. HEAVY METALS (NICKEL AND LEAD), HYPOXIA, AND LIVER FUNCTIONS—ROLE OF DIETARY SUPPLEMENTATIONS

Dietary supplementation of protein and other antioxidants including chelators are found to be effective against metal-induced hepatotoxicities. It has been found that metal ions interact with protein in a coordinated manner and chelate. These protein chelator compounds change the toxic characteristics of heavy metals by degrading it. The mode of hepatotoxicities by toxic metals such as nickel and lead are similar with hypoxia exposure. The cell signal pathways of nickel or lead and hypoxia

usually take place through HIF-1α expressions and further manifestation of expression of hepatic VEGF and NOS2. Both nickel or lead and hypoxia exposure induce ROS and inflammatory cytokines and damages hepatocytes, and interestingly, dietary supplementation of antioxidants such as vitamins C or E and high proteins combat the toxicities from these exposures.

6.1 Heavy Metals, Liver Functions, and Dietary Supplementation

The most common therapeutic way to combat heavy metal toxicity is chelation therapy which leads to metal excretion, but chelators themselves have many contraindications. Chelators such as EDTA and meso-2,3-dimercaptosuccinic acid (DMSA) are routinely used against Pb poisoning, but no such chelators are found to detoxify nickel poisoning so far. Hence alternative therapy, especially dietary supplementation, is now gaining momentum against heavy metal poisoning. As per WHO and the US Dietary Supplements Health and Education Act (DSHEA) of 1994, vitamins, minerals, herbs, amino acids, or other food substances additionally supplemented in diets are considered as dietary supplementation.[80] Most of the cases these dietary supplementations are found to be safe for health.[81] Some studies showed that Zn or Se are protective against Pb and Ni toxicity in liver, kidney, and brain. These micronutrients facilitate antioxidant defense mechanisms of metabolically active tissues including liver by acting as cofactor for synthesis of glutathione peroxidase (GSH-Px).[82] One interesting observation is the beneficial role of iron supplementation in metal exposure. In presence of dietary supplemented iron, it competes with other divalent cations derived from metals such as Ni, Cd, or lead at the level of its transporter proteins such as divalent metal transporter-1 (DMT1) and metal transporter protein 1 (MTP1) in the intestine and reduced uptake of these heavy metals.[83] Dietary supplementations of some elements such as calcium or magnesium are also found to be effective against Pb or Ni toxicity. The elements usually decreases heavy metal absorption from intestine or competitively binds with active sites of intracellular metal-binding protein in hepatic tissues and prevent heavy metals such as nickel, cadmium, and lead to exert hepatic tissue damages.[84,85] Some dietary supplementations such as *Allium sativum* Linn (garlic) were found to be hepatoprotective against heavy metals such as nickel and chromium VI.[86] Garlic has been found to be effective against heavy metal toxicities in liver through a number of mechanisms, such as scavenging radicals, increasing glutathione levels, increasing the activities of enzymes such as glutathione S-transferase and catalase, and inhibiting cytochrome p4502E1. Studies of Vimal and Devaki[87] showed that allicin (diallyl thiosulfinate) which is the main biological active compound derived from crushed garlic is highly protective against Cr VI– or lead-induced hepatic lipid peroxidation. Garlic also contains a number of amino acids that are required for the formation of an enzymatic antidote to free radical pathology, which is created by various pollutants including heavy metals. Cysteine, glutamine, isoleucine, and methionine found in garlic help to protect the liver cells from such free radical damage.[88,89] Raw garlic extract can effectively protect the body from metal toxicity. Garlic contains the highest level of the antioxidant selenium, which affords excellent hepatocellular protection.[89] Vitamin supplementations in diet are extremely popular against heavy metal toxicities as low concentration of vitamins C, B1 and B6 are found to have increased sensitivity toward Cd, Ni, and Pb toxicity in hepatic tissues.[90] It is further observed that vitamins C and E are natural exogenous nonenzymatic antioxidants which prevent liver from oxidative stress by preventing hepatic lipid peroxidation.[47] Besides antioxidant actions, vitamin C also acts as a chelating agent like EDTA against Pb toxicities in hepatic tissues.[91] Experimental study in rats has shown a beneficial effect of vitamin E pretreatment against heavy metals induced an alteration in liver antioxidant defense mechanisms.[20,92] Supplementation of vitamins B1 and B6 were found to be effective in decreasing Pb concentration in liver by reversing ALAD activity. Vitamin B1 facilitates Pb excretion and reduces the Pb toxicity.[93] Other good hepatoprotective agents against heavy metal toxicity are black tea or green tea, grapes, and tomatoes. The bioactive constituent of these edibles are mainly catechins, flavonoids, and polyphenols. These compounds are antioxidants by nature and act as chelators against Pb-, Ni-, or Cd-induced hepatotoxicity.[94–96] Some other plants such as liquorice (*Glycyrrhizae radix*) and ginseng (*Panax ginseng* Meyer) are also found to be effective against Cd-, nickel-, and Pb-induced hepatotoxicities. These plants are routinely used in diet by Chinese, Malaysians, and Africans.[97,98] Currently, some probiotics such as *Lactobacillus rhamnosus*, *L. plantarum*, and *Bifidobacterium longum* are found to be effectively neutralizing heavy metals in vitro. Besides this, these probiotics also act as antioxidants. Probiotic such as *L. plantarum* CCFM8610 is capable to reduce intestinal absorption of heavy metals and reduces metals deposition in liver and reversing hepatocellular damages due to heavy metal toxicities.[99,100] Another dietary option against heavy metal–induced hepatotoxicity is use of algae as it contains good amount of vitamin C, vitamin E, carotene, etc. which help to reduce heavy metal–induced oxidative stress.[101] High dietary supplementation of protein was also found to be effective in liver metabolism against nickel-induced toxicities.[6]

6.2 Hypoxia, Liver Function, and Dietary Supplementation

It has been found that supplementation of vitamins C and E on hypoxic rats improves hepatic glutathione level as compared with only hypoxia-exposed rats. It may be due to antioxidant vitamins C and E protect thiol status in the liver from hypoxia

injuries.[20] Antioxidant vitamins such as vitamins C and E also protect hepatocellular reduction of GSH-Px due to hypoxia exposure by decreasing phospholipid hydroperoxides in the cell membrane and prevent further lipid peroxidation.[20] These two antioxidant vitamins usually conjugate with GSH-Px and are able to decrease phospholipid hydroperoxide in liver to inhibit lipid peroxidation.[102] Results show that vitamin C and vitamin E are oxidized by ROS and RNS generated by hypoxia exposure in liver tissues. During combating with these TOS and RNS, tissue produce superoxide, hydroxyl, peroxyl, and nitroxide radicals, as well as nonradical reactive species such as singlet oxygen, peroxynitrite, and hypochlorate. These scavenging actions truly prevent lipid peroxidation, DNA and protein damage in liver. These antioxidants further enter into mitochondria and guard it from oxidative stress–induced damages. It must be noted that mitochondria of living cells including hepatocytes generate lots of intracellular ROS, and vitamins C and E supplementation defend mitochondrial genome.[103] Study shows that hypoxia exposure leads to decrease of hepatic concentration of vitamin C which may be due to overutilization of vitamin C by the liver tissues to counteract altered oxygen microenvironment in liver.[11] It was noticed that supplementation of vitamin E modifies altered oxygen-sensitive VEGF protein expression in hepatic tissues of hypoxic rats which may be through NOS2. In addition to direct cellular oxidant injury by hypoxia exposure, ROS and RNS may constitute signals regulating by either protein function through reversible oxidation and/or nitrosation of protein SHs or gene expression, in the hepatic sinusoid. Normally nitric oxide (NO) is synthesized by NOS2 gene expression and produced RNS in liver due to hypoxia-exposed low-oxygen microenvironment. Such low-oxygen microenvironment in hepatocytes, Kupffer cells, and endothelial cells generate redox-sensitive transcription factor NF-Kβ.[104] Study also revealed that the hepatoprotective effect of vitamins C and E under conditions of hypoxia appears to be due to its influence on the functional activity of adrenal glands. It was reported that these antioxidant vitamins enhanced noradrenaline-mediated activity in hypoxia through an iodoacetic acid–sensitive pathway.[105]

6.3 Heavy Metals, Hypoxia, and Liver Functions—Dietary Supplementation

Heavy metals such as nickel or lead and hypoxia exposure in liver tissues damage its integrity and develop hepatic malfunctions through ROS and RNS regulatory system. Interestingly, the toxic manifestation for both heavy metals and hypoxia in hepatic tissues are common by nature, i.e. increasing production of ROS and RNS subsequently increase expression of HIF-1 α. It was found that HIF-1α which expresses in tissue exposed to hypoxia as adaptive mechanism is important to regulate metabolism in liver and kidney.[106] Furthermore, it is noticed that HIF-1 plays an important role to develop fatty liver and hepatic fibrosis. It is also noticed that hypoxic area in fibrotic liver due to heavy metals or chronic hypoxia exposure localized with VEGF expression in hepatocytes and HSCs.[107]

The possible mechanism by which vitamins C and E counteract HIF-1α transcription factor expression may be through regulating/inhibiting ROS formation and indirectly controlling over production of RNS**. Hypoxia- and heavy metal treatment–induced HIF-1α gene transcription actually facilitate VEGF gene expression in hepatocytes to improve adaptability against chronic sustained hypoxia and metal-induced cellular hypoxia in physiological system.[108] Reports suggested existence of a feedback mechanism between ROS production and HIF-1 α in metabolically active tissues, although this link is a complex phenomenon which involves oxidative phosphorelation in response to hypoxia or heavy metals**.[109] Furthermore, it is noticed that oxidative phsophorylation in metabolic tissues modulate not only ROS but also oxygen redistribution and consumption by interfering HIF-1α degradation pathways and over expression of endogenous antioxidant enzymes.[110]

6.3.1 Heavy Metals, Hypoxia, Vitamin C and E Supplementation—Liver Histopathology

The author's laboratory shows histopathological changes in nickel- and lead-treated rat liver with or without supplementation of vitamin C and E (Fig. 26.7A–G).

Fig. 26.7C and D shows the effect of vitamins C and E supplementation on nickel-treated rat liver histopathology. It shows that the hepatic parenchymal tissue architecture is maintained normal, which is composed of numerous hexagonal to pyramidal "*lobules*." Each lobule consists of a central vein from which the hepatic plates radiate outward toward the portal areas; three to five portal triads are located at the periphery of the lobule containing branches of bile duct, portal vein, and hepatic artery; and occasional mononuclear cells. Cords of hepatocytes and blood-containing sinusoids radiate from central vein to the peripheral portal triads. The hepatocytes are large having well-defined cell borders with mild variation in cellular size and shape. The nuclei are round, regular, and vesicular with one to two prominent nucleoli. The cytoplasm is eosinophilic and hypergranular. The portal area appears mildly enlarged with mild proliferation fibrous tissue. The sinusoidal space appears normal with moderate number of Kupffer cells. Central vein shows features of mild dilatation and congestion. No foci of fatty change/hyaline change/degeneration/necrosis/inflammatory reaction are found in vitamins

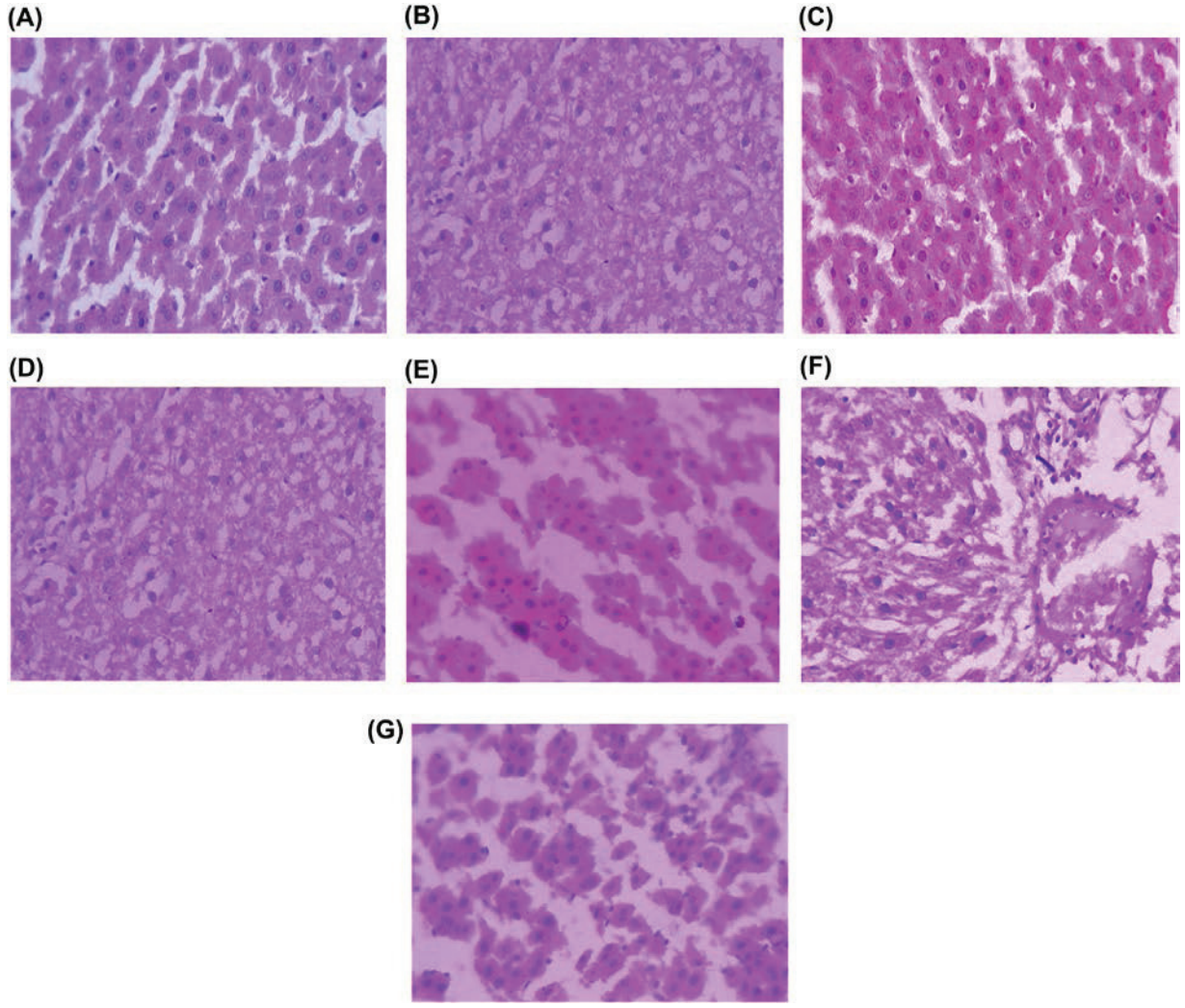

FIGURE 26.7 Normal (A), nickel sulfate– (B), nickel sulfate with vitamin C– (C), nickel sulfate with vitamin E– (D), lead acetate– (E), lead acetate with vitamin C– (F), and lead acetate with vitamin E– (G) treated rat liver histopathology (45×).

C and E supplemented nickel-exposed rats. Results clearly indicate an improvement of hepatic architecture in nickel- or lead-exposed rats supplemented with either vitamin C or vitamin E. Fig. 26.7F shows hepatic parenchymal tissue with mild distortion of "lobular" architecture which is consisting of a central vein, hepatic plates, and portal areas containing branches of bile duct, portal vein, and hepatic artery in vitamin C–supplemented lead treated rat. The hepatocytes are large having ill-defined cell borders with mild variation in cellular size and shape. The nuclei are round, regular, and vesicular with one to two prominent nucleoli. The cytoplasm is vacuolated to clear type with decreasing eosinophilia containing micro/macro vesiculations. There are mild foci of fatty change and ballooning degeneration and necrosis of hepatocytes in zone 3 (centrilobular) areas. The portal area appears mildly enlarged with mild proliferation fibrous tissue with infiltration of mixed acute and chronic inflammatory cells. The sinusoidal spaces are variably widened with pronounced increase in number of Kupffer cells. Central vein shows features of dilatation and congestion. Fig. 26.7G shows near normal architecture of liver parenchyma. Hepatocytes appear normal with sinusoidal spaces that appear normal with moderate number of Kupffer cells. Cytoplasm is eosinophilic and central vein appears normal. Results indicate vitamin E is a better protector than vitamin C against lead-induced hepatotoxicities.

The study on experimental rats in the author's laboratory shows the liver histopathology in hypoxia-exposed and vitamins C and E–supplemented hypoxia-exposed rats (Fig. 26.8A–D).

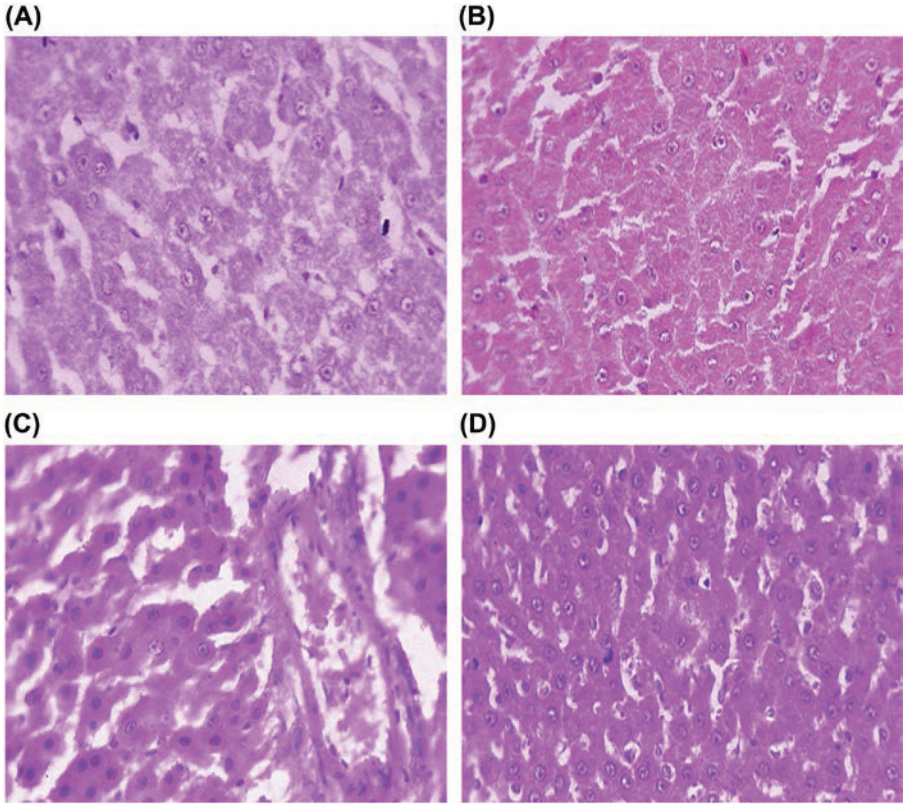

FIGURE 26.8 Normal (A), hypoxia exposed (B), hypoxia exposed with vitamin C– (C), and hypoxia exposed with vitamin E–supplemented rat liver histopathology (45×).

Fig. 26.8B shows hypoxic liver architecture. The central veins are lined by endothelial cells surrounded by a ring of collagen fibers. The sinusoids are lined by both endothelial cells and Kupffer cells both of which have inconspicuous flattened nuclei and ill-defined cytoplasmic margins. The hepatocytes are polygonal in shape with well-defined borders and appear to be little swollen with mild narrowing of the sinusoidal spaces. Microscopic profile shows normal architecture of liver parenchyma maintained with mild narrowing of the sinusoidal spaces. Cytoplasm is more eosinophilic and hypergranular. Fig. 26.8C showed vitamin C shows normal architecture of liver parenchyma is maintained. Mild narrowing of the sinusoidal spaces with portal triad shows mild proliferation with mild thickening of basement membrane of the blood vessels. No obvious significant changes are noticed. In the case of vitamin E–supplemented hypoxic rat liver, normal architecture of liver parenchyma is maintained but hepatocytes appear to be little swollen with mild narrowing of the sinusoidal spaces. The nucleus is single, is round, and has a fine chromatin pattern with zone 1 to 2 clearly defined amphophilic-prominent nucleoli (Fig. 26.8D). Fig. 26.9A shows effect of vitamin C supplementation on hypoxia-exposed nickel (Ni)-treated rat liver. The experimental studies from the author's laboratory on histopathology of liver clearly indicate near normal architecture of liver parenchyma. Hepatocytes appear normal, and sinusoidal spaces appear also normal with moderate number of Kupffer cells. Cytoplasm is appeared to be eosinophilic. Histopathology also indicates normal central vein with normal portal triad. Results show beneficial effect of vitamin C on nickel-treated hypoxic rat liver as compared with rats without vitamin C supplementation (Fig. 26.6A). In case of vitamin E supplementation on nickel-treated hypoxic rats, mild distortion of "lobular" architecture of liver parenchyma and large hepatocytes with mild variation in cellular size and shape are observed (Fig. 26.9B). Furthermore, liver histopathology reveals that cytoplasm is vacuolated to clear type with decreasing eosinophilia containing micro- and macrovesiculations. There are foci of fatty change and ballooning degeneration and necrosis of hepatocytes in zone 3 (centrilobular) areas and mild proliferation of portal area with fibrous tissue with infiltration of mixed acute and chronic inflammatory cells. Variable widening of sinusoidal spaces are also seen (Fig. 26.9B). Although results indicate a relative beneficial effect of vitamin E supplementation on nickel-treated hypoxic rat liver when compared with nickel-exposed hypoxic rats (Fig. 26.6A), it looked relatively less beneficial when compared with vitamin C–supplemented nickel-treated hypoxic rats (Fig. 26.9A).

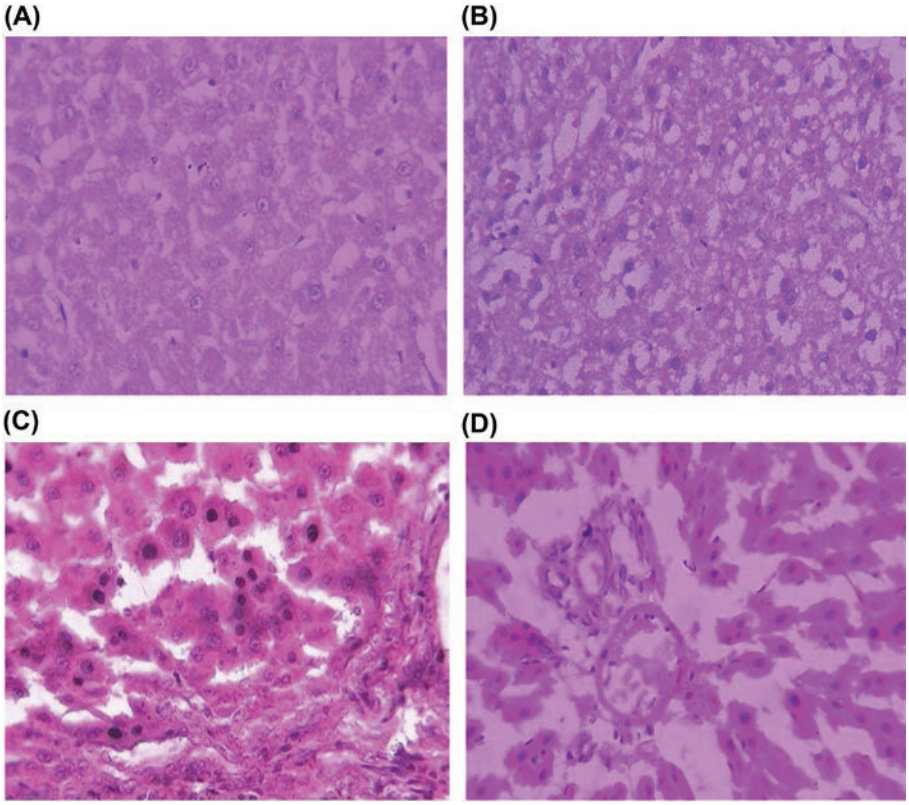

FIGURE 26.9 Nickel and hypoxia exposed vitamin C– (A) and vitamin E– (B) supplemented; lead and hypoxia exposed vitamin C– (C) and vitamin E– (D) supplemented rat liver histopathology (45×).

In authors' laboratory, liver histopathology of lead (Pb)-treated hypoxic rats supplemented with vitamins C and E was also done. Results show a normal architecture of liver parenchyma with mild swollen hepatocytes. Mild narrowing of the sinusoidal spaces was also observed. Portal triad shows mild proliferation with mild thickening of basement membrane of the blood vessels. There were no foci of fatty change or necrosis or inflammatory reaction in histopathology of liver observed (Fig. 26.9C). Fig. 26.9D shows the liver histopathology of Pb-treated hypoxic rats supplemented with vitamin E. Mild distortion of "lobular" architecture of liver parenchyma and vacuolated cytoplasm with decreasing eosinophilia containing micro-and macrovesiculations is noticed. Furthermore, mild proliferation of portal area with fibrous tissue with infiltration of mixed acute and chronic inflammatory cells is also found. Central vein shows features of dilatation and congestion. Results definitely indicate a relative beneficial role of vitamin E supplementation on liver histopathology in lead (Pb)-treated hypoxic rats as compared with lead-treated hypoxic rats without vitamin E supplementation (Fig. 26.6B).

7. CONCLUSION

It may be postulated that heavy metals such as nickel (Ni) or lead (Pb) cause serious cellular damages including hepatocellular damages. Interestingly, chronic sustained hypoxia also induces hepatotoxicities. The molecular mechanisms involved in both the cases are similar by nature.

Heavy metals such as nickel or lead induce hypoxia over expressions of HIF-1α in hepatocellular environment which will be followed by generation of ROS and further expression of VEGF and NOS2 gene in liver. This overexpression of HIF-1α also alters hepatic glycolytic pathways by changing Glut1, LDH, and PFR genes. All these changes lead to hepatocellular damages (Fig. 26.10). Dietary supplements, especially antioxidants such as vitamins C and E are found to be beneficial as they suppress either metal- or hypoxia-induced hypoxia gene expressions in hepatocytes.

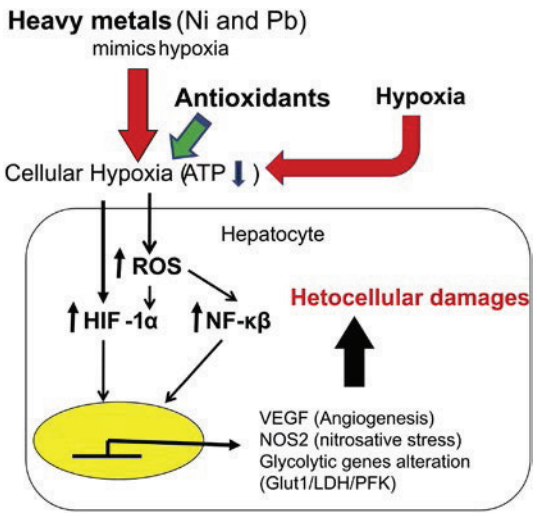

FIGURE 26.10 Heavy metal– and hypoxia-induced molecular mechanisms of hepatotoxicities. *Red arrow* indicates toxicities and *green arrow* indicates protection.

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Dietary Interventions in Liver Disease

Foods, Nutrients, and Dietary Supplements

Edited by Ronald Ross Watson and Victor R. Preedy

Dietary Interventions in Liver Disease: Foods, Nutrients, and Dietary Supplements provides valuable insights into the agents that affect metabolism and other health-related conditions in the liver. It provides nutritional treatment options for those suffering from liver disease. Information is presented on a variety of foods including herbs, fruits, soy, and olive oil showing that changes in intake can change antioxidants and disease-preventing nonnutrients, affecting liver health and/or disease promotion. This book serves as a valuable resource for biomedical researchers who focus on identifying the causes of liver diseases as well as food scientists targeting health-related product development.

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- Addresses the most positive results from dietary interventions using bioactive foods to impact liver disease, including reduction of inflammation and improved function.

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