INTRODUCTION

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Industrialization world over is contributing to an increasingly polluted environment loaded with varieties of chemicals including metals. Humans and animals are exposed to these xenobiotics and metals routinely. The toxicity of a metal depends on its inherent capacity to adversely affect any biological activity (Kusal, 2002). Vadodara, a highly industrialized city of Gujarat, has its share of evnironmetnal contaminations and, the threat of environmental pollution looms large over the horizon. Vadodara has become a mega industrial city with giant complexes like GSFC, IPCL, Refinery, GSFC Polymers, ONGC, Heavy Water Plant, Gujarat Dyestuff Industries, Indian Dyestuff Industries, ABS Plastics, Novino Batteries, besides many medium and small scale industries catering to the production of H-acid, dyes, intermediates, thionylchloride, vinly sulphone, various chemicals, fertilizers, pharmaceuticals etc. The increased discharge of industrial effluents into river Mahi led to the construction of a 56 km long effluent channel passing through 24 villages of Vadodara and Bharuch districts and ultimately terminating into the Mahi estuary (Sharma, 1995). The industrial effluents contain toxic chemicals and other pollutants, inspite and despite of treatment from a common treatment plant. The villagers along the effluent channel are continuously pilfering the effluent channel water for irrigating their land and growing vegetables and cereal crops. Continuous process of irrigation, year in and year out, is likely to pollute the soil, the vegetation and even the underground water bodies (Ramanchandran, 2003). A previous environmental impact assessment study from this laboratory had studied the metal load of vegetables grown in and around Vadodara. This study has documented heavy metal content in these cereals

and vegetables in the range of 3-20 times more than the recommended value of World Health Organization (Sharma,1995). This poses serious threat to the health of people consuming these vegetables and cereals. The above study had clearly recorded Chromium (Cr), Cadmium (Cd) and Nickel (Ni) contents to be higher in the cereals and vegetables. Despite the fact that adverse health effects of heavy metals are being increasingly realized, exposure to heavy metals continues and is ever increasing in some areas.

Cadmium is one of the priority pollutants and is widely present in the environment of Vadodara. Cadmium is the 48^{th} element and a member of group 12 in the periodic table of elements. The most common oxidation number of cadmium is +2 and is a heavy metal. Soluble cadmium salts can accumulate and result in toxicity to the kidney, liver, lungs, brain, testis, heart and central nervous system (Stohs and Bagchi, 1995; Valko *et al.*, 2005). Cadmium can cause osteoporosis, anemia, non-hypertrophic emphysema, irreversible renal tubular injury, eiosinophilia, anosmia and chronic rhinitis (IARC, 1993). Cadmium itself is unable to generate free radicals directly, however, indirect generation of various radicals involving the superoxide radical, hydroxyl radical and nitric oxide has been reported (Galan *et al.*, 2001). The mechanism of toxicity of cadmium can be multifactorial and because of its carcinogenic properties it has been classified as number I category carcinogen by International Agency for Cancer (IARC, 1991).

Chromium, another widely used industrial chemical is known to cause many systemic injuries including DNA damage, lipid peroxidation, enzyme inhibition, cytotoxicity and mutagenesis and the major mechanism of action is due to generation of free radicals

(Stohs and Bagchi, 1995). Chromium is the 24th element of the periodic table. Chromium exists in a series of oxidation states with a valence from -2 to +6; the most important stable states are 0 (elemental metal), +3 (trivalent) and +6 (hexavalent). Trivalent and hexavalent compounds are thought to be the most biologically significant. Cr (III) is an essential dietary mineral in low doses. It is required to potentiate insulin and for the normal glucose metabolism. After entering into the body from an exogenous source, Cr (VI) is rapidly taken up by the erythrocytes after absorption and reduced to Cr (III) inside the cell, thereby generating high amounts of free radicals.

Nickel is another environmental contaminant which after entry into the body targets organs like kidney, lung, spleen, liver, heart and testis. Nickel is the 28th element of the periodic table. It is a silver-white metal found in several oxidation states, ranging from -1 to +4. However, the +2 oxidation state is the most common form of nickel in biosystems. Experimental data suggest that oxidative stress may be important in nickel-induced carcinogenesis. Nickel produces rather low but measurable levels of free radicals in cells (Bal and Kasprzak, 2002). Much of the toxicity of nickel may be associated with its interference on physiological processes regulated by magnesium, zinc, calcium and manganese (Coogan, 1989). Formation of reactive oxygen species and oxidative stress are also related with the ability of Ni to form DNA adducts, cause DNA strand breaks and chromosomal aberrations, induce lipid peroxidation and carcinogenesis (Stohs, 1995; Valko *et al.*, 2005).

In nature, no individual is exposed to a single or an isolated toxicant, but to a combination of toxicants depending on the characteristics of the area. Hence, this study

was employed to see whether a combination of the three heavy metals namely chromium (Cr), cadmium(Cd) and nickel(Ni) would have additive, synergistic or antagonistic interaction on each other as compared to their action singly. It is being increasingly realized that transition metals by acting as catalysts in the oxidative reactions of biological macromolecules contribute to oxidative tissue damage. Multifactorial mechanisms have been implicated in metal induced toxicity and, one of the well known mechanisms suggested is, the formation of metal induced reactive oxygen species (ROS) (Nuran *et al.*, 2001). Oxygen free radicals are involved in a wide variety of normal cellular functions but, they can be both essential as well as highly toxic to cellular homeostasis (Freeman and Crapo, 1982). Oxygen free radicals are generated as byproducts of normal cellular metabolism; however, several conditions are known to disturb the balance between oxygen free radical production and cellular mechanisms. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. The burden of ROS is largely counteracted by an intricate antioxidant defense system including enzymatic scavengers (SOD, GPx, GR, and Catalase) and non-enzymatic antioxidant defense (glutathione, flavanoids, Vit C and Vit E).

The primary defense against ROS is provided by Superoxide Dismutases (SODs) which are a group of metalloproteins found in both prokaryotic and eukaryotic cells. SODs are thought to dismutate O_2^- via a ping-pong mechanism whereby the transition metal prosthetic group is reduced by O_2, forming H_2O_2 (Fridovich, 1974). Catalase (CAT), a manganese or heme-containing enzyme, functions to rapidly dismutate H_2O_2 to H_2O and O_2 (Krinsky, 1992). Gluthatione (GSH) dependent system plays a vital role in the

antioxidant defense mechanism in animals. The reduced tripeptide GSH is a hydroxyl radical and a singlet oxygen scavenger which participates in a wide range of cellular functions (Halliwell and Gutteridge, 1989; Deneke and Fanburg, 1989). Reduced glutathione acts as a hydrogen donor and as such is a substrate for key antioxidant enzymes including the Se-dependent glutathione peroxidase (GPx) and glutathione-S-transferases (GSTs). GPx removes hydrogen peroxide and organic hydroperoxides (Ahmad, 1995; Fridovich, 1998) while, GSTs catalyze conjugation reactions between glutathione and ROS-damaged cellular components. Glutathione reductase (GR) functions to recycle glutathione, converting the oxidized form of glutathione (GSSG) back to GSH using the reducing power of NADPH (Ahmad, 1995). Lipid peroxidation (LPO) is an indicator of oxidative stress and hence assessed to gauge the degree of damage to biological membranes. Regulation of antioxidant enzyme activity in eukaryotes may be influenced by such factors as age, hormonal status, organ specificity, and amount of cofactors present (Harris, 1992).

Increased knowledge about the action of reactive oxygen species and oxidative stress has led to intensive search for new and more effective substances which prevent extreme development of oxidative stress and, these substances are called antioxidants, scavengers, trappers or quenchers. Melatonin (N-acetyl-5-methoxytryptamine) was discovered by Dr. Aaron B. Lerner (Lerner, 1958). The main role of endogenous melatonin involves receptor mediated biological rhythm synchronization (Konecna et al., 2001). Melatonin is shown to have significantly broader actions including oncostatic effects, immune system stimulation and anti-inflammatory functions (Blask *et al.*, 2002; Cuzzocrea and

Reiter, 2002). More recently, melatonin has been identified as a powerful direct free radical scavenger (Tan *et al.*, 1993, 2002) and indirect antioxidant (Reiter *et al.*, 2000). Melatonin seems to function via a number of means to reduce oxidative stress (Reiter *et al.*, 2003). The available experimental evidences support its actions as a direct free radical scavenger (Hardeland *et al.*, 1993; Allegra *et al.*, 2003), as an indirect antioxidant by stimulating antioxidant enzymes (Reiter *et al.*, 2000; Rodriquez *et al.*, 2004), as an agent capable of stimulating the synthesis of glutathione (an essential intracellular antioxidant) (Urata *et al.*, 1999), as an up-regulator of transcription and production of mRNA specific to SOD, CAT, GPx and GR (Karwonik *et al.*, 2000) and, as an agent capable of inhibiting the activity of nitric oxide synthetase (NOS) (Bettahi et al., 1996). Melatonin is highly lipoidal in nature (Costa et al., 1995) but is also somewhat aqueous (Shida et al., 1994). The widespread subcellular distribution of melatonin may allow it to interact with all molecules, thereby reducing oxidative damage to molecules in both the lipid and aqueous environments of the cell. Its ability to cross all biological membranes and the fact that it does not require a receptor for this action does enhance the anti-oxidative property of melatonin. Hence, the present study was taken up to assess the ROS generation and oxidative stress induced by single or a combinational mixture of the three heavy metals, as well as to assess whether, melatonin can act as antioxidant protector against the heavy metal mediated oxidative damage of liver and kidney tissue *in vivo* as well as *in vitro* using two different human liver cell lines.