INTRODUCTION

In the entire cultural history of mankind, one of the worst ecological crises is the problem of environmental pollution. Pollution of the environment concerns the contamination of the ecosystem which again causes instability, disorder, harm or discomfort to the physical systems or living organisms. The heightened concern for reduction of environmental pollution that has been occurring over the past 20 – 25 years has stimulated interest of scientists in research on the toxicology of heavy metals. While the toxic effect of these substances is a widespread concern in the modern industrial context, Man has succeeded in poisoning himself with them repeatedly throughout recorded history. One historian contends that the fall of the Roman Empire was hastened by chronic lead poisoning experienced by the ruling classes who had water conducted through lead plumbing and drank wine from goblets which had lead/alloy composition. Minamata, Japan disaster, claimed entry into the lexicon of toxicology fifty years ago as a definition of methyl mercury poisoning but deposited legacy of unsolved problems that still endures. Again Itai-itai disease (literally: "ouch-ouch" disease) one of the four big pollution diseases of Japan, was the first documented case of mass cadmium poisoning in the world in Toyama Prefecture, Japan in 1950. The cadmium was released in the rivers by mining companies in the mountains.

The blame of ecological crisis of environmental pollution has been put on different things of which one major factor is the pollution due to metals in the environment. Metals are an intrinsic component of the earth’s crust and are omnipresent in the environment in varying concentrations in soil, water, air and all biological matter.
With rapid industrialization and unrestricted usage; metals with toxic levels are now everywhere and affect everyone on planet earth. Moreover, metals are also released into the environment from a wide spectrum of anthropogenic activities such as smelting of metallic ores, industrial fabrication and commercial application of metals, agro-chemical, and pesticides as well as burning of fossil fuels. These metals are redistributed in the biosphere through food chain causing chronic ailments due to bio-magnification. Virtually all metals can produce toxicity when ingested in sufficient quantities, but there are several which are especially important because either they are so pervasive, or produce toxicity at such low concentrations. They have become a major cause of illness, aging and even genetic defects. The physiological influence of metals on the organisms of human and animals is conditioned by the nature of metal, by the type and amount of compounds (Danielyan, 2010). Another significant effect of environmental pollutants is their role as etiological factors in several chronic diseases.

When speaking of heavy metals one generally means lead, mercury, cadmium, iron, copper, manganese, arsenic, nickel, aluminum, silver, and beryllium. Toxic metals comprise a group of minerals that have no known function in the body and, in fact, are harmful. Today mankind is exposed to the highest levels of these metals in recorded history. Many heavy metals are essential for plants and animals when present in growing medium in low concentrations; they become toxic only when a concentration limit is exceeded. Metal phytotoxicity has been known for more than a century. However, the interest in this field has developed only recently. The capacity of the plants to survive on soils that contain high concentrations of certain toxic metals has fascinated ecologists and plant physiologists for decades. Again the number of persons suffering from subclinical
metal poisoning is believed to be several million. A large portion of the cases are in
developed countries but the urban areas of developing countries have become ‘hot-spots’
of metal pollution, and the population of such countries is particularly susceptible to
environmental toxins. As a global problem, the potential health effects of metallic
hazards should be a matter of public health concern, especially if the emissions of toxic
metals into the environment continue at the current rate.

Among the myriad environment pollutants, cadmium merits special reference as a
toxic element. Cadmium is an important environmental global toxicant. Through decades
people have been exposed to this heavy metal through geogenically-contaminated
drinking water. In various countries inadequate disposal of nickel-cadmium batteries is
also adding to the problem of environmental contamination. The health hazards of
cadmium have attracted increased attention since the rock-soil-plant-animal-man
relationship became better known, and when it was realized that low doses are
cumulative over a long period of time and may endanger the general population. The
elimination of cadmium from the body takes place at an extraordinary low rate, so that
the metal accumulates in the body, primarily in the liver and kidneys. The elevated
cadmium level in groundwater is a new public concern in India. Study reveals that the
groundwater area, in the tea garden belt of Darrang district, Assam, India is highly
contaminated by cadmium. Again Son beel, one of the largest wetlands in Assam, is
found to be highly contaminated with cadmium. (Borah et.al., 2009).

Cadmium is a transitional metal that exists in different oxidational or transitional
states (Donald et.al., 1996) it occurs in rock erosion and abrasion and volcanic eruptions
(Jarup et.al., 1998; 2000) fossil fuels and particularly non-ferrous mining and metal
industries (Friberg et. al., 1986). The compound has varying degrees of solubility, absorption and toxicity (Falks et. al., 1990). Major sources of cadmium intoxication are mining and smelting, metal coating, welding, incineration of refuse, fossil fuels, use of sewage sludge as fertilizer, smoking of tobacco and many others. Residential sites may be contaminated by municipal waste or leaks from hazardous waste sites.

In general heavy metals produce their toxicity by forming complexes or "ligands" with organic compounds. These modified biological molecules lose their ability to function properly, and result in malfunction or death of the affected cells. The most common groups involved in ligand formation are oxygen, sulfur, and nitrogen. When metals bind to these groups they may inactivate important enzyme systems, or affect protein structure. The primary biochemical mechanism of action of cadmium toxicity is that it stimulates and binds to many proteins and non protein sulfydryl groups, macromolecules and metallothionein, leading to inhibition of numerous enzyme systems (Aprioku and Obinime, 2009). These modified biological molecules lose their ability to function properly, and result in malfunction or death of the affected cells. For a metal to have its biological effect, be it beneficial or toxicological, it must interact and cross the cell membrane; enter the cell where it then can bind either reversibly or irreversibly to a cellular target and thereby alter specific biochemical processes (Paul, 2010). They can elicit a number of immunomodulatory effects leading ultimately to an enhanced susceptibility of immune cells to microbial agents and the appearance of neoplastic diseases and autoimmune phenomena. Heavy metals provoke changes in the function(s) of immune cells (Degange et.al., 2006). Cadmium (Cd) exposure has been associated with a wide range of toxic effects including nephrotoxicity, hepatotoxicity, alterations in
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bone formation, reproductive physiology and immune system (Koller, 1998; Nordberg, 1996; Waalkes et al., 1999).

Blood serves as a good medium for bringing in contact the peripheral immunocompetent cells after intoxication with heavy metals, as well as, with the resident cell repertoire fixed in different lymphoid organs of the body. Monocyte-macrophage lineages are essential component of the host defense system. Macrophages, the active phagocytic cells, are capable of ingesting and digesting exogenous antigens such as whole microorganisms, insoluble particles, injured and dead host cells, cellular debris and activated clotting factors (Owais, Gupta, 2005). Neutrophils remain dedicated primarily to the destruction of invading microbes while macrophages act both as phagocytic as well as antigen presenting cells (Benjamini et al., 2000). As such macrophages play a crucial and connecting link between innate and adaptive immune system.

Studies reveal that the heavy metals are not only toxic for the organisms but may also modulate immune responses. The immunomodulatory activity was proved *in vivo* and *in vitro* model systems (Krocova et al., 2000) Cadmium being a potent immunotoxicant, affects both humoral and cell mediated immunity (Pathak and Khandewal, 2009). Many cell types are involved in humoral and cell mediated immune response, in which macrophages, T-cells and B-cells play a crucial role. Long term intake of small amounts of cadmium can lead to increased tolerance to its acute toxicity. However it is a matter of fact to investigate the tolerance that may be associated with a reduced chronic toxicity as well (Gebel, 2001). Additional question arise when we consider several factors suspected to modulate the long-term toxicity of cadmium *in*
variables that may either enhance or suppress the environmental genotoxicity and
carcinogenicity of the metal. Hence the evaluation of toxic potentials of cadmium is
important for the risk assessment of human beings ordinarily exposed to this heavy metal
in the environment.

In general, the consequence of any toxic response depends upon the particular cell
or organ affected the severity of the damage and the capability of the impaired cells or
tissue to recover from the assault (Gardner, 1984). Spleen in one of the most important
organs of the immune system which if affected adversely may result in
immunocompromised stage of an organism and as such splenic macrophages were
targeted as the cells of investigation. Owing to their scavenging and phagocytic functions,
spleen macrophages are regarded to be important in the induction and maintenance of
both innate and acquired immune defense mechanisms. Again peritoneal macrophages
were chosen for investigation as being the wandering ones that patrol the whole body.
Any alteration in these two particular cell lines may greatly alter the balance of the
immune system.

While the toxic effects of cadmium have wide-spread concern in the modern
industrial context, knowledge of their effects on the immune system of an organism is of
immense importance. Owing to the established roles of macrophages in immune defense,
we planned to study, whether cadmium can affect these major immune cells. Although
the actual immunocompetence of animals on toxic metal exposure undergoing a chronic
inflammation has been directly studied, there was no doubt that these trace metals are
deeply involved in the development and maintenance of normal immune functions which
may be due to alteration of cellular activity. At present cadmium (Cd)-induced immunotoxicity and the mechanisms involved have not been fully elucidated (Dong et. al., 2001). No clear information is available regarding the effect of cadmium on these immunocompetent cells. A wide range of toxic effects has been associated with cadmium (Cd) exposure in mammals. However, the physiological factors that modulate these effects have received limited attention (Pillet et.al., 2006). Previous studies have shown that heavy metals exert marked immunomodulatory effects; however the exact mechanism of their influence on the immune system is unclear.

The objective of the present study was to determine any alteration in the immune functions in cadmium exposed mice and its underlying mechanism. Whether cadmium modulates surface molecules to alter interactions of macrophages with tissue surfaces and affect mitigation of the inflammatory responses was a potent question. Cell functions like cell adhesion, morphological alteration (by scanning electron microscopy), chemotactic migration, phagocytosis, intracellular killing, in vivo clearance, enzyme release were assayed to determine the non-specific immune functions in splenic macrophages. Very little is known about macrophage function during cadmium intoxication followed by *Staphylococcal* infection. Hence *in vivo* bacterial clearance assay from blood and spleen was also performed to characterize macrophage function during cadmium intoxication followed by *Staphylococcal* infection. The objective of the present study was also to observe whether cadmium exposed mice could efficiently clear *in vivo* bacterial load. Emphasis was also given on immunomodulatory effects of the heavy metals cadmium in mice. A preliminary study on any change at the molecular level was made by means of DNA fragmentation assay. Humoral functions were addressed by plaque forming and
agglutination assays; while delayed type hypersensitivity (DTH) responses as determined by foot-pad swelling addressed the cellular immunity status. The proinflammatory aspect was investigated by means of various cytokine and hypersensitivity tests. Suitable in vitro models would facilitate further investigation of the critical chemical species cadmium involved in immunomodulatory process. In the present study attempt has been made to use in vitro immunobiological parameters as surrogate markers for the toxicity of cadmium on murine immune system. Here an attempt has been made to know the effect of cadmium on the immunocompetent cells with special reference to splenic and peritoneal macrophages.