CHAPTER – VII

CONCLUSION
The current in vivo study demonstrates that exposure of male mice to cadmium chloride resulted in alteration in morphology induces DNA fragmentation of testicular macrophages; reduced phagocytosis index of testicular macrophages indicate that cadmium treated groups are more prone to infection, as they cannot phagocytose efficiently and so cannot clear out the invading microorganism. Cadmium intoxicated testicular macrophages were not able to kill the intracellular Staphylococcus aureus competently as compared to control. Cadmium chloride exposure in mice also decreased the testicular weight. These toxic effects induced by cadmium were in agreement with the effects of other toxic metals that are also sulphydryl group modifiers (Acharya et al., 2003). The results of this study strongly suggest that exposure to the heavy metal cadmium in the form of cadmium chloride had severe effects on testicular macrophages as a result chemotactic migration was also effect. The capacity to migrate specially towards an inflammatory stimulus was found to be reduced significantly after exposure to cadmium. Activated macrophages produce various antimicrobial and cytotoxic substances which help in phagocytosis of microorganism. During the study it was found that oxygen dependent as well as oxygen independent cytotoxicity was greatly affected due to cadmium exposure. Further study explains that cytokine release was also hampered due to continuous exposure of cadmium which ultimately decreases the total activity of macrophage. Hence, it can be summarized that the toxic potential of cadmium is overtly manifested in the testes and this may bear particular significance in heavy metal induced infertility.

In vivo studies offer a general overview of the physiologic function of an organism. However, multiparametric factors are a hindrance to the elucidation of the underlying mechanisms in such studies. To facilitate such elucidation, in-vitro
studies are important and help emisage a simplified and minimalistic model of the toxicity profiles of environmental toxicants.

The *in vitro* study shows morphological alteration along with DNA fragmentation in cadmium treated (*in vitro*) group. It also indicates an impairment of the microbial capacity due to compromised phagocytosis process, intracellular killing, chemotactic migration and a reduction of inflammatory responses. This is due to decrease of toxic oxygen and NOS species in the presence of cadmium ions. It was observed that release of enzymes like MPO was also affected due to *in-vitro* cadmium treatment. Thus, both the *in vivo* and *in vitro* studies are in accordance to each other. Moreover loss of functions of the testicular macrophages upon cadmium exposure entails a study of the effect of this metal on reproductive parameters and related oxidative stress. The final part of the study thus, focuses on semenological parameters, male accessory gland marker (fructose), testosterone level and antioxidant status.

The third and final part of the study shows decrease in sperm count, sperm motility and increase in sperm abnormality (such as detached head and / or coiled tail) in adult male albino mice treated with cadmium chloride. Historical alteration and other, abnormalities in the sperms could be due to reduced spermatogenesis in the testes of the treated mice. Similar results were reported by Neveen et al. (2007), where exposure of adult male mice to cadmium chloride significantly decreased sperm counts, total number of sperms per mg of testis, daily sperm production efficiency. In addition, it was also reported that cadmium has a detrimental effect on testicular function (stages of spermatogenesis) that could result in reduced sperm production leading to reduced male fertility (Bench et al. 1999). There are definitive scientific evidences (Jeremy et al., 2002), that testosterone elevations were associated with proper reproductive function. Cadmium intoxication not only
affects spermatogenesis but also the male accessory organs assess from levels of marker of seminal vesicle (fructose). Thus the alterations in both these parameter can correlated with reduced testosterone levels.

Also, the increased oxidative stress resulting from cadmium intoxication in testicular tissue might be responsible for poor semen quality, testicular damage and impairment of fertility. Moreover, it has been reported that the oxidative stress affects the sperm cells via interfering with the membrane fluidity which is the main factor for sperm motility and fusion with the oocyte (Kim and Parthasarathy 1998, Aitken, 1995). Toxic effect of cadmium on testes is known to deplete glutathione and protein – bound sulphydryl groups, which results in enhanced production of reactive oxygen species (ROS) such as superoxide ion, hydroxyl radicals and hydrogen peroxide (Waisberg et al., 2003). In the current study, mice exposed to cadmium chloride showed a significant reduction in the activity of antioxidant enzyme (SOD and catalase) and a concomitant enhancement in lipid peroxidation (LPO). In conclusion, it can be considered that cadmium intoxication greatly alters normal reproductive function as well as immune status of the testes, thus emphasizing that concern should be directed towards limiting the inadvertent incorporation of cadmium in human-consumed products.

Besides emphasizing on the immunomodulatory properties of cadmium, the present work also highlights the effect of cadmium even in an immunoprivileged niche, namely the testis. Thus, the effect of cadmium exposure on testicular macrophages not only portray a potent mechanism of immunosuppression as well as reproductive toxicity, but also give clues to occurrence of an immune induced infertility in mice.