SUMMARY
“Reproductive toxicity includes adverse effects that inhibit sexual function and fertility in males and females. It also takes into account any short or long-term direct exposure *in vitro* or *in vivo* of chemicals or agents that are recognized to possess such toxic effect on reproductive organs or cells of reproductive origin. Leydig cells constitute a significant majority among interstitial cell population located in between the seminiferous tubules. These cells support steroidogenesis and synthesize the male sex hormone, testosterone. The reproductive function of sperm production occurs within the seminiferous tubules of the testis, the process of which is entirely dependent on the testosterone produced from the Leydig cells and also from FSH, the other hormone, secreted from the pituitary gland. Besides the pituitary endocrine loop, the recent understanding has recognized many other local regulatory control loops of regulation of spermatogenesis well coordinated by various intracrine and paracrine factors secreted from the tubular or interstitial compartments.

Since Leydig cells are found morphologically juxtaposed with the interstitial macrophages with cellular processes extending deep into the indentations of macrophages, it is believed that macrophages do possess some regulatory control over Leydig cell steroidogenesis probably through their secretions and vice versa. Macrophages are rich source of reactive oxygen species (ROS), recognized to aid many physiological processes only at lower concentrations. But, H$_2$O$_2$ being a ROS species has been found to inhibit hCG induced testosterone production in isolated Leydig cell even at lower concentrations and raising the concentration does impact cell viability and induction of apoptosis as well. Considering the fact that Leydig cell dysfunction in clinical situations is not only associated with diminished steroidogenesis but also with reduced fertility and hypospermatogenesis, in the present work we attempted a targeted approach of inducing adverse effects on Leydig cells both *in vitro* and *in vivo* and later explored ways to ameliorate the effect by appropriate intervention.

For *in vitro* studies, H$_2$O$_2$ was selected as the ideal toxicant having implications of local production and release from macrophages and possessing ability to leach out from the source of origin and affecting the survival and function in the adjacent target cells. In the first experimental section, the effect of the oxidant H$_2$O$_2$ (100 µM) was investigated particularly on the issue of survival and induction of apoptosis in Leydig cells and the extent the effects could be
countered by supplementation either with a recognized antioxidant N-acetyl cysteine (NAC) or Eugenia jambolana (EJE) fruit pulp extract, a gift from Prof. S.B. Sharma, University College of Medical Sciences, Delhi. The molecular mechanism of modulation in the expression of various marker proteins of apoptosis was studied in detail with respect to H₂O₂ treatment and also during improvement following simultaneous supplementation with NAC or EJE. In the subsequent section, the process of H₂O₂-induction of apoptosis was further investigated to identify the preferential channelization; either extrinsic or intrinsic pathway of metazoan apoptosis. siRNA to caspase 8 and -9 was utilized for the purpose and based on the findings it was concluded that H₂O₂-induced apoptosis in Leydig cells is initially channeled through extrinsic pathway later possibly extending to other pathways.

The third experimental plan for inducing adverse effect on Leydig cells in vivo was realized by administering cisplatin, a therapeutic drug commonly used for treatment of cancer. Cisplatin is recognized to induce reproductive toxicity in clinical as well as animal studies and also reported to affect Leydig cell survival and function. Since EJE has shown to possess antioxidant properties during intervention studies in vitro, as detailed above, it was again investigated in vivo in comparison with NAC in the CIS treated rats. EJE supplementation was seen more potent than NAC ameliorating the cisplatin induced adverse effects on Leydig cells. Even the functional Leydig cells and the sensitivity of the cells to hCG induced testosterone production demonstrated a significant improvement. The above studies have confirmed the antioxidant potential of the plant extract and its usefulness in conditions of cisplatin mediated reproductive toxicity. However, data on safety related aspects need to be examined before the findings are applied clinically for the benefit of the mankind.”