Leydig cells represent the primary source of production of testosterone, the male sex hormone which is essential for the development of male phenotype. Impaired testosterone production affects both sexual development and fertility. But, testosterone is not only vital for sustaining the reproductive function but also for many other physiological systems of the body as well. Beyond its traditional role as primary source of androgen production, Leydig cells also secrete other steroids and non-steroidal factors. It is now well recognized that these other products play a critical role in modulating physiological control mechanisms responsible both for steroidogenesis and sperm production. Understanding the local control mechanisms of Leydig cell function is likely to reveal more information about the signals that mediate cross compartmental communication between the Leydig cells and seminiferous epithelium and also between diverse cell populations within the interstitial space.

Impaired Leydig cell function has been considered as a marker for testicular dysgenesis syndrome (TDS) which includes a whole range of male reproductive disorders leading ultimately to infertility. Diminished spermatogenesis is characteristic of lower testosterone levels with higher LH concentrations. Even compromised Leydig cell function has inherent poor response to human chorionic gonadotropin (hCG) stimulation. On the other hand, exposure of normal testes to agents that damage spermatogenesis, like irradiation or treatment with chemotherapeutic drugs etc. affects Leydig cell function equally in the absence of any underlying “congenital” lesion representing TDS. Leydig cells are positioned in close proximity of macrophages in the interstitium which signifies the fact that reactive oxygen species (ROS) secreted by macrophages could also play a significant role on Leydig cell steroidogenesis. Based on the work from our laboratories, \( \text{H}_2\text{O}_2 \), a ROS species is reported to affect hCG-stimulated testosterone production in Leydig cells \textit{in vitro}. It is thus pertinent that adult Leydig cell dysfunction needs to be managed appropriately and conditions responsible for the same
also require careful assessment and analysis. Therefore, in the present study we proposed to affect Leydig cell function through H₂O₂ exposure and later tried to curtail the damage and revive the function through intervention with a plant extract with antioxidant properties and analyzed the benefits in comparison with a recognized antioxidant. The extract has been reported for its use as an anti-diabetic, anti-inflammatory agent but its antioxidant potential has been investigated in detail for the first time in the present work.

The whole work consists of two independent sections comprising the adverse effects of H₂O₂ on Leydig cell survival and function along with associated molecular mechanisms followed by amelioration by the antioxidant, N-acetyl cysteine (NAC) or the fruit pulp extract *Eugenia jambolana* intervention, both *in vitro* and *in vivo* conditions.

In the first chapter (Section I: Experimental Plan Chapter I), the work has been mainly concentrated on studies in which isolated rat Leydig cell are utilized. Leydig cells were exposed to different concentrations of H₂O₂ and a right concentration (100 µM) of the compound was finally selected which maintained >80% viability but induced apoptosis in about 50% of the target population. The intervention with NAC or EJE was tried along in order to find out the extent of counteraction supporting target cell survival under identical conditions. H₂O₂ induced apoptosis in the Leydig cells which was associated with increase in lipid peroxidation and simultaneous decline in the activities of antioxidant enzymes. Even total glutathione and total antioxidant capacity demonstrated a significant depletion following H₂O₂ exposure. It not only stimulated nitric oxide (NO) formation but also upregulated the gene expression of nitric oxide synthase, the enzyme responsible for synthesis of NO. However, all the adverse effects were found effectively countered with either EJE or NAC supplementation. Cell survival with the extract was found comparable to similar effects by N-acetyl-L-cysteine. Co-treatment of the extract helped in the downregulation of caspase-3 and poly-ADP-ribose polymerase resulting a significant reduction in Leydig cell apoptosis induced by H₂O₂. Upstream marker proteins of extrinsic (caspase 8, Fas, FasL) and intrinsic (caspase 9) pathway of metazoan apoptosis were identically down-regulated. The Bcl-2 family of proteins, though, remained unaffected. The extract also positively modulated the other
marker proteins like c-Jun NH2-terminal kinase, p38, Akt, nuclear factor k-B, c-Fos, cellular FLICE-inhibitory protein, cyclooxygenase-2 and p53.

As it was learned that H\textsubscript{2}O\textsubscript{2} exposure up-regulates most of the marker genes responsible for apoptosis, attempts were made in the subsequent Chapter (Section I: Experimental Plan Chapter II) to understand the channelization of pathway in H\textsubscript{2}O\textsubscript{2}-induced apoptosis of primary Leydig cells \textit{in vitro} using siRNA as a tool. Expression of apoptotic marker genes, caspase 8, -9, -3 and polyadenosine ribose polymerase was subsequently investigated using a concentration (250 µM) of H\textsubscript{2}O\textsubscript{2} post 1 h exposure. Incubation with siRNA (20 nM) either for caspase 8 or -9, inhibited their individual expressions and activity. The inhibition efficiency using siRNA was comparable with post- or pre- H\textsubscript{2}O\textsubscript{2} treatment of cells. Like siRNA, \textit{Eugenia jambolana} (100 µg/ml) plant extract too, effectively countered over-expression of all upstream apoptotic marker proteins. Silencing expressions of caspase-8 but not -9 through siRNA leads to a profound inhibition of caspase-3 implying that H\textsubscript{2}O\textsubscript{2} induced Leydig cell apoptosis is preferably channeled through extrinsic and later possibly extending to other pathways. The above findings further confirmed the contention that in primary testicular cell culture model presently employed; H\textsubscript{2}O\textsubscript{2} is considered as the ideal exogenous agent for up-regulating gene expressions in a shortest possible time and has relevance for wider use in similar experimental culture conditions with limited cell longevity.

The third chapter (Section II: Experimental Plan Chapter III) is an extension of the \textit{in vitro} study demonstrating the application of use of EJE \textit{in vivo} and the beneficial effects of the same intervention under conditions of chemotherapeutic drug-induced reproductive toxicity. Cisplatin (CIS) was selected because of its reported reproductive toxicity even after one time administration. Rats were administered cisplatin (5 mg /kg bw, single dose) either alone or along with EJE (25 mg/kg bw, every alternate day) or NAC (150 mg/kg bw, every 3\textsuperscript{rd} day) for seven days. Following sacrifice on day 8, significant alterations in serum LH, FSH and testosterone were observed. In contrast, EJE or NAC supplementation to CIS-treated rats, effectively improved FSH, LH and testosterone levels. There was also a significant rise in functional Leydig cells which was associated with upregulation of expression
in 3β-HSD protein and transcript levels. Testicular oxidative stress demonstrated a significant decline with restoration of total antioxidant capacity or glutathione levels. Augmentation in the activities and expressions of antioxidant enzymes, SOD, catalase, glutathione–s-transferase (GST), glutathione reductase (GR) was seen. CIS induced apoptosis was not limited to germ cells but extended to include Leydig cells too. Both NAC as well EJE intervention were able to contain the rise in apoptotic induction by effective modulation of apoptotic markers in the extrinsic, intrinsic and other pathways of metazoan apoptosis. The study findings establish the potential of EJE as a therapeutically better antioxidant than NAC and may be further explored for use in clinical studies in curtailing the adverse effects of anticancer drugs on testicular function.

In conclusion, the present work demonstrated that the fruit pulp of *Eugenia jambolana* has antioxidant properties which act as an antidote against the toxic effects as a result of direct exposure of Leydig cells to oxidants or indirectly through chemotherapy. Such cytoprotective effect also augments Leydig cell steroidogenesis since hCG-induced testosterone production rises significantly in these cells following EJE supplementation.