5. Summary and conclusion

Modern world is suffering from the problems of population explosion and infertility. WHO standardise the normal value of sperm parameter. Infertility is considered when the parameters are below the standard range. Oligospermia (low sperm concentration), abnormal sperm structure (teratospermia), low sperm motility (asthenospermia) are the associated terminology with infertility. There are so many causative factors for the onset of infertility such as testicular failure, low semen volume, erectile dysfunction, endocrine abnormality, ejaculatory failure and environmental causes etc. Oxidative stress plays a prime role in male reproduction. Free radicals are required for sperm maturation, acrosomal reaction, capacitation, sperm-oocyte fusion but on the other side, excessive free radical production damages the sperm DNA by imposing oxidative stress via activation of stress induced caspase mediated apoptosis which leads towards the onset of infertility.

Nutraceutical plays a crucial role on maintaining the balance between antioxidant level and free radical generation. Nutraceutical also can able to inactive the ROS at cellular as well as molecular level. Lycopene is bright red carotenoid mainly available in tomato, water melon, papaya etc. It is effective in quenching the singlet oxygen. Lycopene is a promising effect on managing oxidative stress induced pathological abnormalities. This Ph.D. work has been designed to focus on the role of lycopene on CPA induced male infertility. The outcome of the experiments is presented in summarized manner below:

Experiment No. I The experiment was performed to ensure the development of infertile animal model. For this purpose, CPA was administered at the dose of 3.0 mg/ 0.5 ml distilled water/ 100 g body weight/ day for 30 days. In this concern, body weight, reproductive organ weight, sperm count, motility and viability were assessed. Activities of androgenic key enzymes such as testicular Δ^5, 3β-HSD and 17β-HSD, levels of testicular

cholesterol were also analysed. Antioxidant profile such as activities of catalase, SOD was determined to focus the free radical generation capacity of CPA. Toxicity profile includes GOT and GPT in serum level was also determined. Histometric analysis includes seminiferous tubular diameter. **Result focused a significant deterioration in all the parameters which confirmed that CPA at the above mentioned dose can able to develop an infertile animal model successfully.**

Experiment No. II The experiment was carried out to determine the minimum dose of lycopene having maximum revival efficacy. In this concern, three different doses of lycopene such as 0.75 mg, 1.5 mg, 3.0 and 4.5 mg / 0.5 ml tween-80/ 100 g body weight/ day for 30 days have been incorporated to the CPA induced infertile animals. Spermatogenic profile such as sperm motility, viability, count, HOS, acrosomal status, androgenic key enzyme activities i.e. Δ5, 3β-HSD and 17β-HSD in testicular tissue were performed. Testicular cholesterol, serum testosterone levels were also determined. Antioxidant profile such as catalase, peroxidase activities and CD level was also assessed. Toxicity profile includes total protein, albumin, globulin were also conducted. Quantification of seminiferous tubular diameter was also performed. Result focused that among all the doses, **1.5 mg dose of lycopene showed most promising efficacy in connection to the rectification of the CPA induced infertility because the lower dose was unable to execute its remedial property and the higher doses saturated the concern receptor of the phyto-molecule.**

Experiment No. III Determination of the threshold duration of the lycopene treatment in connection with the rectification of CPA treated reproductive abnormalities in male rats was the aim of this experiment. For this purpose, 15 days, 30 days and 45 days duration was considered as the duration of lycopene treatment. Parameters such as sperm motility, count, acrosomal study, NCD, body weight, reproductive organ weight were assessed. Androgenic

key enzyme activities such as Δ5, 3β-HSD and 17β-HSD were analysed. Simultaneously, serum testosterone, testicular cholesterol, seminal vascular fructose levels were determined. Antioxidant profile includes catalase, SOD were also performed. Toxicity profile such as GOT, GPT, uric acid, urea, creatinine, BUN were analysed in this experiment. Result highlighted that among all the durations, **30 days duration was considered as the maximum duration of lycopene treatment to correct the CPA induced male reproductive complications.**

Experiment No. IV This experiment was carried out to focus the reversible and irreversible nature of the lycopene treatment to determine the sustainability of the revival efficacy of lycopene after its withdrawal for 15 day, 30 days and 45 days to the CPA treated testicular hypo-function. Sperm motility, count, acrosomal study was conducted to confirm the sustainability of the lycopene treatment. Effect of lycopene withdrawal was further studied by analysing the activity of Δ5, 3β-HSD and 17β-HSD at testicular level. Oxidative stress profile was determined by measuring the activity of testicular catalase, SOD, GST, peroxidase and levels of CD and TBARS. Lipid profile also assessed that includes triglyceride, LDL, VLDL and HDL. **After statistical analysis of the obtained data, the result revealed that lycopene may exerts its remedial activity by lowering the CPA induced oxidative stress related spermatogenic problems in irreversible manner up to 30 days.**

Experiment No. V The experiment was performed to delineate the molecular mechanism behind the rectification of CPA treated male infertility through genomic pathway after oral administration of lycopene. For this experiment, gene expression and protein expression study of androgenic key enzymes, antioxidant enzymes, and apoptotic markers of testicular tissues were performed through qRT-PCR and western blot analysis respectively.

To confirm the DNA damage after CPA administration and its rectification by lycopene administration was confirmed by conducting comet assay and ISEL study. Flow cytometric analysis of sperm viability and sperm mitochondrial integrity was also performed. Result focused that **lycopene can able to manage the CPA induced hypo-testicular dysfunction cum infertility by altering ROS induced apoptosis.**

Experiment No. VI The study was carried out to find out the direct effect of lycopene on antioxidant enzymes and androgenic key enzymes in connection with the rectification from CPA induced infertile condition. The testes treated with CPA at the potent dose in *in vitro* manner. In this concern, activities of androgenic key enzymes such as Δ5, 3β-HSD and 17β-HSD and antioxidative enzymes such as testicular catalase, SOD, GST and peroxidase were analysed. Result focused a non significant difference in the activities of androgenic key enzymes between CPA treated group and CPA+ lycopene treated group. But the activities of the antioxidant enzymes were recovered significant towards the vehicle treated control which signifies the **lycopene has a direct role managing CPA induced male infertility either destroying the free radicals or by elevating the antioxidant enzyme activities.**

Experiment No. VII Determination of toxicity effect of lycopene if any was the main aim of this experiment. For this, different toxicity markers were adopted. Serum level of GOT, GPT, albumin, globulin were assessed. Serum protein profile such as urea, BUN, uric acid, creatinine, total protein levels was determined. Similarly, serum lipid profile such as LDL, VLDL, HDL and triglyceride levels was also analysed. Activities of ALP and ACP in liver and kidney were also determined. Result revealed that lycopene at the dose of 1.5 mg/ 0.5 ml tween 80/ 100 g body weight/ day did not exert any toxic effect to the blood, kidney and liver.

Experiment No. VIII This experiment was performed to assess the capacity of lycopene to retrieve the fertility ability to the CPA treated infertile rats. In this concern female rats were allowed to met with the male rats treated with CPA and CPA+ lycopene treated groups (details were mentioned in the experiment section). Implantation sites were found in the both uterine horns of the female rats mated with the lycopene treated male rats where as no implantation sites were observed in the uterine horns of the female rats mated with the CPA treated ifertile male rat. Result established that lycopene may have the potentiality to regain the fertility capacity by recovering free radical induced sperm damage.

On the basis of the above experiments, following conclusion may be drawn

- Cyproterone acetate at the dose of 3.0 mg/ 0.5 ml distilled water/ 100 g body weight for 30 days can develop an infertile animal model.
- Among 0.75 mg, 1.5 mg, 3.0 mg and 4.5 mg/ 0.5 ml tween-80/ 100 g body weight doses 1.5 dose showed maximum recovery in connection with the CPA induced male reproductive abnormalities.
- 30 days duration of lycopene treatment among 15 days and 45 days was considered as the threshold duration to achieve maximum recovery form the CPA induced male infertility.
- Lycopene can able to sustain its revival efficacy up to 30 days of withdrawal of lycopene treatment to the CPA treated testicular hypo-function in rat.
- Lycopene may improve the anti-apoptotic and antioxidant gene expression by lycopene-gene interaction by which apoptosis related sperm DNA damage can be checked.

- *In vitro* study revealed that lycopene has direct role on the antioxidant enzymes by which stress can be minimised and stress induced sperm damage can be prevented.
- Lycopene has the capability to regain the fertility ability .
- No toxic effect was found after oral administration of lycopene to the CPA induced infertile animals.