SUMMARY AND CONCLUSION

MPA + DHT COMBINATION

In this study, the contraceptive efficacy of a combination of progestin (MPA) and an androgen (MPA + DHT) has been evaluated in sexually mature adult male rats. The contraceptive efficacy of this treatment was assessed in relation to the biochemical and morphological alterations in epididymal sperm, reproductive and vital organ metabolism and functional integrity. Recovery status of these hormonal regimen was also studied for 60 and 90 days after cessation of the hormonal regimen.

The parameters studied were the fertility rate, whole body and organ weights as well as the cauda epididymal sperm profiles like sperm motility, sperm count, sperm viability, sperm morphology and acrosomal integrity. The biochemical parameters of sperm viz. hyaluronidase, acrosin and superoxide dismutase (SOD) were estimated in order to assess their function. Succinate dehydrogenase (SDH), adenosine triphosphatase (ATPase), protein and sialic acids were quantified in caput and cauda epididymides to assess their functional integrity and metabolism. Testicular biochemical tests viz. 3β and 17β hydroxysteroid dehydrogenases (HSDS), acid phosphatase (ACPase) cholesterol and protein were studied. In liver glycogen, phosphorylase and ascorbate levels were assessed respectively. Haematological parameters such as haemoglobin content, blood cell counts, blood SOD and serum parameters like testosterone, cholesterol, protein and transaminases were also analysed. The histology of testis was also done in normal, treated and withdrawal groups of animals.
Weekly intramuscular injections of medroxyprogesterone acetate (MPA, 2.5 mg) along with dihydrotestosterone (DHT, 500 µg) for 60 days, were found to have no significant alterations in the whole body and organ weights. The data revealed a depletion in sperm reserves as a result of this treatment, in addition to loss of sperm motility in the cauda epididymis. Morphological defects and alterations in viability of sperm were also observed. Significant reduction in sperm SOD levels implied changes in sperm plasma membrane permeability and was correlated with loss of sperm viability and sperm motility. The sperm acrosin system and hyaluronidase levels in treated rats decreased and were related to loss of their fertilizing capacity. All these changes brought about an altered sperm function thereby impairing the fertility rate of the treated animals. But, normal mating rate was noted.

The testicular biochemical tests indicated a reduction in their levels. Histology of testis exhibited spermatogenic activity arrest in most of the seminiferous tubules in MPA + DHT treated rats and is accounted for the loss of its weight due to intratesticular androgen deficiency.

The changes in the epididymal biochemical parameters like succinate dehydrogenase (SDH), adenosine triphosphatase (ATPase) and sialic acids also generated alteration in its microenvironment leading to maturational defect of the sperm as a result of local androgen deprivation state.

Moreover, no significant changes were detectable in haematological parameters like haemoglobin, SOD and blood cell counts. Clinical chemistry parameters were within the normal range. However, insignificant elevation in serum
transaminases and defective glycogen metabolism were observed stating probable dysfunction of the liver. Other serum cholesterol, protein and testosterone levels did not exhibit much alterations.

The recovery data after discontinuation of the hormonal regimen for 60 and 90 days revealed gradual restoration of all affected parameters with respect to sperm viz., sperm concentration, sperm motility, sperm viability and sperm morphology in the epididymis. The fertility rate of these animals were also comparable to control group. Similarly, normal function of reproductive and vital organs were also observed in withdrawal groups. Serum chemistry parameters were also within the normal levels. Thus, it was suggestive that the observed side effects seem to be transient and were completely reversible upon the withdrawal of the treatment.

In conclusion, a combination of MPA + DHT would be feasible for male steroid contraception, if proper spacing regimen and doses are known. Moreover, non-aromatizable androgens do not undergo aromatization leading to undesirable side effects.

PLANT PRODUCTS

This section reports the antifertility effects of two plant extracts, one from pericarp of unripe fruits of *Balanites roxburghii* and other from the whole plant of *Phyllanthus amarus* in adult male mice. These extracts possessed good spermicidal effect at 2% concentration *in vitro*. Oral feeding of 70% alcoholic extracts of *Balanites roxburghii* and *Phyllanthus amarus* at different doses viz., 100, 250, 400 and 500 mg/kg body wt. for 45 days were studied for their
antifertility action. Out of these, a dose of 500 mg/kg body wt. dose was selected as it exhibited a better antifertility effect. The contraceptive efficacy of these extracts at 500 mg/kg was assessed with respect to the biochemical and morphological alterations in the epididymal spermatozoa, as well as reproductive and vital organ metabolic integrity. Their reversibility for 30 and 45 days was also investigated.

The parameters studied with these two plant products were fertility rate of the animals, the whole body and organ weights, cauda epididymal sperm profile like sperm motility, sperm count, sperm viability and sperm morphology by eosin staining. Biochemical parameters like SDH, ATPase, protein in the epididymides and testicular biochemical parameters such as SDH, alkaline phosphatase (ALKPase), cholesterol and protein were estimated. In addition, toxicity study in regard to haematological parameters like haemoglobin content, blood cell counts, serum testosterone, cholesterol, protein and transaminases levels were also assessed. The histology of the testis, the cauda and caput epididymides were also carried out in normal, treated and withdrawal groups of animals.

Daily oral feeding of 70% alcoholic extracts of *B. roxburghii* and *P. amarus* at a dose of 500 mg/kg to mice for 45 days, had no effect on the whole body and organ weights. The sperm motility and sperm count were significantly decreased in the cauda epididymis affecting the spermatogenesis. The percentage of the viable sperm also declined with an increased number of non-viable forms by Trypan blue staining after 45 days of feeding of these extracts. Eosin staining method showed elevated abnormal sperms in extract fed mice.
However, there was no alteration in mating rate but a significant decline in the fertility rate of these animals was noticed.

The biochemical enzyme activity in the epididymides like SDH and ATPase were decreased which showed alterations in the epididymal milieu causing maturational defects of the sperm. The testicular parameters like SDH, and ALKPase cholesterol were also affected revealing changes in its metabolic and functional integrity. The histological features of these tissue were not significantly affected. As the extracts had no effect in circulating testosterone levels, it is likely that these extracts have no effect on hypothalamo-pituitary gonadal axis. But it is possible that these are effective at target site level which requires further elucidation by assessing 5α-reductase and androgen receptor levels as well as intratesticular and epididymal androgen levels.

The toxicological studies revealed no significant changes in haematological parameters like haemoglobin content, blood cell counts and serum transaminase levels which indicated non-toxicity of these extracts. However, Phyllanthus extract resulted in a slight increase in serum cholesterol levels.

Recovery studies for 30 and 45 days of cessation of extracts feeding, the biochemical parameters, sperm profiles as well as the fertility potential of the animals restored gradually and comparable to the pretreatment levels. Thus, these extract possessed reversible antifertility activity with no side effects.

In conclusion, these preliminary data on both of these extracts revealed promising results for the development of an ideal, oral and reversible male
contraceptive agent of plant origin. Further studies are called for in this direction.

**FUTURE LINE OF WORK**

Based on the work embodied in the present thesis, the following investigations could be carried out to ascertain the contraceptive efficacy of the steroid hormones and plant products.

**HORMONAL COMBINATION**

1. The exact hormone pill dose and spacing regimen studies are to be decided so as to produce functional sterility with free of side effects.

2. Sperm functional tests such as hamster/rat oocyte penetration (HOP/ROP) test should also be done.

3. Sperm morphological and ultrastructural changes need to be studied.

4. Sperm free radical contents are also needed to monitor as these are related to plasma membrane permeability changes.

5. Androgen target organs such as testis, epididymis and accessory organ metabolism and functional integrity will also be studied using specific markers in addition to the vital organs.

6. The tissue anatomical features will also be ascertained.
7. Blood differential counts need to be done.

8. Serum protein hormones, testosterone, estradiol, dihydrotestosterone and other hormones are to be assayed.

9. Serum lipids and protein profiles need to be measured to evaluate the feasibility of these dose regimens.

10. Testicular spermatokinetics will be done to prove the effects of steroids at specific cellular level.

**PLANT PRODUCTS**

11. The alcoholic extracts need to be further analyzed and to be isolated for identifying the active principle(s) in them.

12. Estogenicity of these extracts will be done.

13. LD50 values need to be estimated.

14. These antifertility effects could be further studied in order to achieve complete sterility without side effects.

15. Enrocrinological and androgen receptor studies will be undertaken to elucidate the mechanism of action of these products.
16. 5α-reductase levels are to be done to further understand their actions.

17. Chromosomal aberrations in testis and bone marrow will also be carried out.

18. Micronucleus studies to assess toxic effect of extracts will be called for.

19. The contraceptive efficacy will be done in other species to check species specificity.

20. Cross checking of these products in other laboratories should be done.