CHAPTER V

SUMMARY AND CONCLUSION
HORMONE COMBINATION

In this study, the contraceptive efficacy of a combination of progestin (MPA) and testosterone enanthate (TE) has been evaluated at a dosage of 20 and 40 mg/kg b. wt./month respectively for four months in sexually mature adult male rats. The long-term effect of this treatment was also assessed in relation to the biochemical and histological alterations in the testis, epididymis and vas deferens, and biochemical changes in prostate and seminal vesicles in adult male rats. Recovery studies of this hormonal regimen were also studied in these rats.

The parameters studied were whole body and organ weights, cauda epididymal sperm profiles viz. motility, count and viability. Fertility rate was also evaluated. Biochemical estimations of 3β and 17β hydroxysteroid dehydrogenases (HSDs), succinate dehydrogenase (SDH), alkaline phosphatase (ALKPase), acid phosphatase (ACPase), total ascorbic acid (TAA), cholesterol and proteins were estimated in testis to study its functional integrity and metabolism. Levels of ATPase, proteins and sialic acid were quantified in cauda epididymides. In the vas deferens, succinate dehydrogenase (SDH), ATPase and protein levels were estimated. Haematological parameters viz. haemoglobin content, blood cell counts, serum levels of transaminases, proteins, cholesterol, total lipids and testosterone were analyzed to evaluate long-term toxicity of the drug combination. The histology and histocytometry of various reproductive organs were also carried out in normal, treated and withdrawal groups of animals.
Monthly intramuscular injections of medroxyprogesterone acetate (MPA) along with testosterone enanthate (TE) for four months was found to have no significant alteration in whole body weight. Organ weights were decreased. The data revealed a depletion in sperm reserves as a result of this treatment in addition to a loss of sperm motility and viability in the cauda epididymis. Alterations in viability of sperm were also correlated with alterations in sperm membrane permeability leading to a loss of their function. All these changes finally brought about an altered sperm function thereby impairing the fertility of the treated rats.

Alterations in the testicular biochemical parameters indicated changed testicular metabolism and function due to an affected intratesticular hormone production. Histology of the testis too exhibited spermatogenic arrest at spermatocyte level in the seminiferous tubules of MPA + TE treated rats and is probably accounted for the loss of its weight as well.

Changes in some epididymal biochemical parameters viz. ATPase, sialic acid and proteins reflected on its altered secretory functions. The altered microenvironment of the epididymis thus demonstrated its adverse effects on sperm maturation as a result of local androgen deprived state. This is further reflected by the histological changes in the region of cauda epididymides. Similarly vas deferens biochemical and histological parameters were also altered due to androgen deprivation. This is further evident by a reduction in serum testosterone levels gradually. But the toxicological studies revealed no significant variation in blood cell counts. Serum parameters also did not indicate much variations. But serum testosterone levels were reduced after four months, as mentioned earlier.
Recovery data after discontinuation of the hormonal regimen for 120 days revealed restoration of all affected parameters comparable to control animals. Thus, it is suggestive that the observed effects seemed to be transient and reversible upon withdrawal of the hormonal regimen.

In conclusion, MPA + TE combination satisfied the criterion for an effective hormonal method of contraception in the male. This combination hence would be feasible for the male if proper spacing regimen and dose studies are known, to achieve consistent azoospermia or severe oligozoospermia state with altered sperm function with no long term side effects.

**PLANT PRODUCT**

This section reports the antifertility effect of plant extract of seed of *Abrus precatorius* in sexually mature adult male mice. Oral feeding of 70% alcoholic seed extract of *Abrus precatorius* at 20 and 40 mg/kg body weight for 45 days were assessed for their antifertility action. Of these, 40 mg/kg body weight dose was selected for withdrawal studies for 90 days as it exhibited a better antifertility effect.

The parameters studied were whole body and organ weights, cauda epididymal sperm profile like sperm motility, count and viability. Fertility rate was also assessed. Biochemical parameters like 3β and 17β HSDs, SDH, ACPase, ALKpase, total ascorbic acid (TAA), cholesterol and proteins were evaluated in testis whereas in cauda epididymides, ATPase, sialic acid and protein were done. In vas deferes succinate dehydrogenase (SDH), ATPase and proteins were estimated and fructose was assessed in seminal vesicle. Toxicity studies in regard to haematological tests like haemoglobin
content, blood cell counts, serum transaminases, proteins, cholesterol, total lipids and testosterone levels were assessed. The histology and histocytometric studies were also done in all experimental groups of animals.

Daily oral feeding of 70% alcoholic extract of *Abrus precatorius* at a dose of 40 mg/kg b.wt for 45 days, had no effect on whole body and organ weights except in the testis and cauda epididymides. The sperm motility and sperm count were significantly decreased in cauda epididymis. The percentage of the viable sperm also declined, leading to a significant reduction in fertility rate of these animals.

The biochemical parameters in testis like 3β and 17β HSDs, SDH, proteins showed a decrease and proteins increased whereas rest of the parameters remained similar to control animals. Sialic acid was also reduced in cauda epididymis showing alterations in the epididymal milieu causing maturational defects of the sperm. The histological changes in testis, distal vas and cauda epididymis were also noteworthy, probably due to androgen deprivation locally. It was supported by reduction in serum androgen level and a fall in seminal vesicle fructose. Thus these alterations in reproductive tissue caused by the extract might be due to direct and indirect mechanisms that affect gonadal hormone production in this study.

The toxicological studies however revealed no significant changes in haematological parameters like haemoglobin content, blood cell counts and serum parameters viz., transaminases, proteins, cholesterol and total lipids indicating non-toxicity of the extract.

During recovery studies for 90 days of cessation of extract feeding, the affected parameters were restored gradually and became comparable to pretreatment levels.
In conclusion, these preliminary data on the extract revealed a promising result for the development of an ideal, oral, reversible contraceptive agent of plant origin in a rodent model. Further studies are called for.

**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 steroid hormones and herbal product.

**HORMONAL COMBINATION**

1. The exact hormone dose and spacing regimen studies are to be decided so as to produce functional sterility without side effects.

2. Sperm function tests such as sperm mitochondria activity index (SMAI), hamster/rat oocyte penetratin (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 be monitored as these are related to plasma membrane permeability changes.

5. Blood differential count needs to be done.

6. Testicular spermatokinetics could be done to prove the effects of steroids at specific cellular level.
PLANT PRODUCT

7. Estrogenicity of extract needs to be done.

8. The alcoholic extract needs to be further analyzed and purified for identifying the active principle(s) in it.

9. Spermatokinetics and sperm function tests need to be carried out in extract fed animals.

10. Endocrinological and androgen receptor studies are to be undertaken to elucidate the mechanism of action.

11. Genotoxic studies are also called for.