SUMMARY AND CONCLUSIONS
CHAPTER V

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HORMONE COMBINATION

In this study, the contraceptive efficacy of a combination of progestin (MPA) and non-aromatizable androgen (DHT) has been evaluated at a dosage of 20 mg and 1 mg.kg\(^{-1}.d^{-1}\) for 90 days respectively in sexually mature adult male rats. The contraceptive efficacy of this treatment was assessed in relation to the biochemical and morphological alterations in the testis, epididymis, spermiogram and vas deferens in adult male rats. Toxicity and recovery studies of this hormonal regimen were also studied in these rats.

The parameters studied were, whole body and organ weights, the cauda epididymal sperm profiles viz., motility, count, viability and acrosomal integrity. Testicular biochemical tests viz. 3\(\beta\) and 17\(\beta\) hydroxysteroid dehydrogenases (HSDs), succinate dehydrogenases (SDH), alkaline phosphatases (ALKpase), acid phosphatases (ACPase) and protein were assessed to study its functional integrity and metabolism. SDH, ALKpase, ACPase, Protein and sialic acid were also quantified in caput and cauda epididymides. In the vas deferens, glycogen phosphorylase and protein levels were done followed by electrophysiological changes of the vas deferens. Haematological parameters viz., haemoglobin content, blood cell counts, serum levels of testosterone, cholesterol, protein and transaminases were also analyzed to evaluate toxicity of the drug combinations. The histology and histocytometry of various reproductive organs were also carried out in normal, treated and withdrawal groups of animals.

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Bimonthly intramuscular injections of medroxyprogesterone acetate (MPA) alongwith dihydrotestosterone (DHT) for 90 days were found to have no significant alteration in whole body and organ weights except in the weights of the testis. The data revealed a depletion in sperm reserves as a result of this treatment in addition to a loss of sperm motility in the cauda epididymis leading to a loss of fertility. Morphological defects and alterations in viability of sperm were also observed, which were correlated with alterations in sperm membrane permeability leading to a loss of their function. The silver nitrate staining of sperm showed acrosomeless defective sperm related to loss of their fertility capacity. All these changes finally brought about an altered sperm function thereby impairing the fertility of the treated animals.

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

Changes in the epididymal biochemical parameters viz. SDH, ATPase, sialic acid and proteins indicated its altered secretory function. The altered microenvironment of the epididymis which 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 regions of the caput and cauda epididymides. But the vas deferens did not exert any significant changes biochemically and histologically. However, the lumen had free of sperms. But contractility pattern of the vas deferens was altered, due to probable changes
in neuro hormonal mechanisms. The toxicological studies revealed that the haemopoietic tissue did not exhibit significant variation as blood cell counts did not reveal much changes comparatively. Other serum cholesterol, proteins and transaminases also did not exhibit much variation indicating normal liver function. Since the serum testosterone levels was not altered, the libido was maintained by this hormonal combination.

Recovery data after discontinuation of the hormonal regimen for 90 days revealed restoration of all affected parameters with respect to sperm profiles viz. sperm motility, sperm viability and sperm morphology in the epididymis. The fertility rate of these animals was also comparable to control groups. Normal functions and histology of reproductive organs viz., testis and epididymis were observed in the withdrawal groups. Thus, it is suggestive that the observed effects seemed to be transient and reversible upon withdrawal of the hormonal regimen.

In conclusion, MPA + DHT combination satisfies the criterion for an effective hormonal method of contraception in the male. Moreover non-aromatizable androgen do not undergo aromatization leading to undesirable side effects like testosterone and its esters. Thus, this combination would be feasible for the male if proper spacing regimen and dose studies are known to achieve complete consistent azoospermia or severe oligozoospermia state with altered sperm function. Further esters of DHT are more preferable than that of DHT as it has a short duration of action.

PLANT PRODUCTS

Antifertility effects of two plant extracts viz. pericarp of unripe fruits of *Balanites Roxburghii* and whole plant of *Phyllanthus amarus* in adult female mice. Oral feeding of
alcoholic extracts of *Balanites roxburghii* and *Phyllanthus amarus* at different doses viz. 100, 250, 500 mg.kg\(^{-1}\).d\(^{-1}\) for 30 days were studied for their antifertility action. Out of these a dose of 100 mg.kg\(^{-1}\).d\(^{-1}\) was selected as it exhibited a better antifertility effect. The contraceptive efficacy of these extracts at this dose level was assessed with respect to the biochemical and morphological alterations in the reproductive organs viz. ovary and uterus including their toxicity. The reversibility for 30 and 45 days was also investigated in these mice.

The parameters studied were whole body and organ weights, cyclicity and fertility rate in these groups. Biochemical parameters like 3β, 17β HSDs, cholesterol, ascorbic acid, glutathione and protein in the ovary and glycogen, phosphorylase, total lipids and proteins in the uterus were done. The spontaneous contractility pattern of the uterus was also carried out to find out their effects. In addition to this, toxicity study in regard to haematological parameters like haemoglobin content, blood cell counts, serum cholesterol, protein and transaminase levels were also assessed. The histology as well as histocytometry of the ovary and uterus was carried out in all the groups for comparison.

Oral feeding of alcoholic extract of *Balanites roxburghii* and *Phyllanthus amarus* each at doses of 100 mg.kg\(^{-1}\).d\(^{-1}\) to mice for 30 days had no effect on whole body and organ weights indicating no effect on general metabolism and growth. However, the extracts fed mice brought about a significant decrease in the uterine weight which could be attributed to the retarded uterine growth due to hormonal imbalance. The extract fed mice too revealed an irregular cyclicity with a predominance of diestrous stage affecting fertility in these animals.
The activities of steroid dehydrogenases in the ovary indicated a reduction that might account for the altered ovarian steroidogenesis leading to an altered hormonal balance. This is further evidenced by an accumulated cholesterol levels and is correlated with the regressive changes in respect to the follicular development observed histologically in the extracts fed mouse ovary.

The uterine biochemical parameters like protein and total lipids were also lowered since the hormonal milieu was altered. These effects were related to the alterations in the histological and histocytometric studies of the uterus. Moreover the spontaneous contractions of the uterus in estrous stage of the extracts fed mice showed a significant increase due to alterations in the neuroendocrine and endocrine regulatory mechanisms. These induced effects seemed to be more in mice fed with Belanites extract than those of others fed with extract of Phyllanthus. However, the toxicological studies revealed no significant change in blood parameters like haemoglobin content, blood cell counts and serum cholesterol, protein and transaminase levels which indicated non-toxicity of these extracts.

After 45 days of cessation of the extract feeding the biochemical parameters of these organs, cyclicity and fertility potential of the animals restored gradually and were comparable to the control levels. Thus the extracts possessed reversible antifertility activity with no side effects except in the contractility pattern in uterus of mice fed with Belanites extract. In conclusion, these preliminary data of both these extracts revealed promising results for development of a herbal product for regulation of fertility in the females. Further studies are called for in this direction.
FUTURE LINE OF WORK

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

HORMONAL COMBINATION

1. Sperm functional tests such as hamster/rat oocyte penetration (HOP/ROP) test should be done.
2. Sperm morphological and ultrastructural changes need to be studied.
3. Sperm free radical contents are needed to monitor as these are related to plasma membrane permeability changes in severe oligozoospermia state.
4. Blood differential counts need to be done.
5. Serum protein hormones, testosterone, estradiol, dihydrotestosterone are to be assayed.
6. Serum lipid and protein profiles need to be evaluated to find feasibility of these drug combination.
7. Testicular spermatokinetics will be done to prove the effects of steroids at specific cellular level.
8. Space regimen studies need to be carried out using various doses of hormonal injections.
9. Long-term studies need to be studied.
10. Esters of DHT are to be explored, as those of androgen esters for use instead of DHT.
PLANT PRODUCTS

1. The alcoholic extracts need to be further analysed to isolate and identify the active principle(s) in them.

2. Antifertility effects of each principle isolated need to be studied.

3. Estrogenicity of the extracts will be done.

4. Endocrinological studies will be undertaken to elucidate the mechanism of action of these products that are potent.

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

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

7. Toxicity studies including histology of various organs are to be carried out.

8. Teratogenicity needs to be done.

9. Reversibility studies of each active principle of the plant product are to be carried out.