CONCLUSIONS

PART 1

From the work presented in this thesis, the following overall conclusions could be drawn:

1. The aqueous 
   *Carpaya sylvestris* seed extract treatment to intact adult male albino rats brought about antifertility effect within 7 days at a dosage of 1 ml/0.1 ml/day/rat, i.e., injections.

2. The treatment caused an androgen-deprived effect to target organs to a variable degree. The cauda epididymides and distal vas deferens were on the whole affected more than other organs.

3. The treatment also manifested variable degree of changes in the histoarchitecture of caput and cauda epididymides and proximal and distal regions of vas deferens.

4. Significant reduction in sperm count and percent motility of cauda epididymal spermatozoa and alterations in sperm morphology of cauda and vas deferens sperm might have contributed towards the zero fertility rate after the treatment.

5. The treatment also brought about changes in the contractile
response of isolated right and left vas deferens of
-treated rats as compared to control.

6. However, no significant changes in the circulating
levels of serum testosterone, FSH and LH hormones were
discerned under treated conditions.

7. Combined extract + ascorbic acid feeding resulted in
the recovery of most of the parameters including organ
weights, indicating the beneficial effects of ascorbic
acid feeding under physiologically altered conditions.
However, this did not interfere with the contraceptive
effect of the seed extract.

8. Withdrawal of 7 day seed extract treatment for 1 and
2+ months restored the normal organ weights, androgenic
parameters, ascorbic acid turnover pattern, fertility
rate, histoarchitecture, contractile response of vas
dererens and hormonal levels indicating that the induced
effects of the extract were transient and reversible.

9. However, throughout the treated conditions, Carica
papaya seed extract treatment did not bring about
reduction in body weight, changes in the weight and
histology of testis, its sperm count, steroidogenic
enzymes (such as 3 β, 17 β hydroxy steroid dehydrogenases)
and its metabolism, indicating that the extract had no
direct effect on the testicular functions.
10. There were no side effects of the treatment.

11. The mechanism of action of the seed extract treatment appears to be due to low target organ response and/or the reduced conversion of testosterone to its potent metabolites. It is also likely that the extract might also alter the hormone-receptor interaction at the target cells and thereby interfere with the hormone action.

12. Hence, it is concluded that *Carica papaya* seed extract is a potential post-testicular male contraceptive whose effects are reversible in nature, and detailed studies on this plant product might pave the way for the development of a so-called 'simple, economical male pill'.

PART II

1. Aqueous *Vincetoma rosse* leaf extract treatment brought about antiandrogenic, antianabolic and antifertility effects in adult male albino rats of proven fertility.

2. The antifertility effects could be induced within a short period of 7 and 15 days, at a dosage of 1 mg/0.2 ml/day/rat, i.m. injections.

3. The leaf extract treatment caused alterations in the organ weights, including pituitary weight, sperm count
and percent motility of cauda epididymal spermatozoa and their morphology, biochemical alterations in caput and cauda epididymides and vas deferens (VDP and VDD) resulting in zero percent fertility rate.

4. The treatment induced significant changes in the histological architecture of testis, epididymides, vas deferens and seminal vesicles.

5. The antiandrogenic effect of the extract was further substantiated from the results of groups IV and V rats where the extract + testosterone treated rats did not show normal levels of some androgen sensitive parameters.

6. However, combined leaf extract + ascorbic acid feeding restored many parameters including organ weights to normalcy, indicating that ascorbic acid helps in the recovery of the antiandrogenic effects of the leaf extract, without interfering with its antifertility effects.

7. Withdrawal of 7 days treatment for 1 and 2-2½ months brought about significant recovery in all parameters including organ weights, except in the weights of seminal vesicles, where a significant increase was noted.

8. Although there occurred no change in the hormonal levels of testosterone, FSH and LH after 7 days treatment, in one month withdrawal group of rats a significant
increase in the testosterone levels and a subsequent fall in FSH levels were noted. This might be due to the slow activity of the extract at the pituitary-gonadal level.

It is concluded that although aqueous *Vinca rosea* leaf extract appears to have potent antiandrogenic and antifertility effects, more detailed studies are required to elucidate the effects of this plant extract on other para-reproductive organs to eliminate the possibilities of other toxic effects.

**FUTURE LINES OF WORK**

The data obtained on the effects of aqueous *Carica papaya* seed extract and *Vinca rosea* leaf extract treatments on adult male albino rats suggests that both these plant extracts are potent antifertility agents whose induced effects are transient and reversible by cessation of treatment. However, the former plant product appears to be more promising for the future, since it is already known that *Vinca rosea* plant alkaloids have antimimetic effects and also exert their effects on the neuroendocrine system. Keeping these facts in mind, some suggestions are made regarding the future lines of work, which could be worthwhile to undertake inorder to establish various facts, as an extension of the present work embodied in this thesis.
1. **CANTICA PAPAYA SEED EXTRACT**:

1. The criterion of ripeness of the fruit from which seeds are taken for extract preparation to be considered on the basis of contents of sugar.

2. Extraction, isolation and purification of the active alkaloids and/or other products of the seeds and pulp and their effects in male rats should be carried out.

3. Studies on the feasible route of administration of the extract, oral pill, injectables etc., depending on its taste, action of proteolytic and salivary enzymes etc., have to be carried out.

4. After the requisite treatment of rats with the extract, the treatment should be withdrawn for a longer period, i.e. four or even more months, until 90-100% regain of fertility rate is attained. The extent of recovery in different animals should also be investigated.

5. The animals which do not respond to withdrawal treatment should be given an exogenous dose of testosterone.

6. The females used for fertility test to be retested by mating them with normal male rats of proven fertility in order to ascertain that the fertility is restored in them.
7. The sexual behaviour (Libido) of treated males caged with normal cycling females should be carefully observed (although in the present study no change in libido was noted).

8. Since the extract treatment brought about significant changes in the metabolism of all androgen target organs to a variable extent it would be worthwhile to study the levels of 5 α- HSD, Δ⁴-5 α-reductase and 3 α-hydroxy steroid dehydrogenase activities in epididymis in control and treated animals according to the method of Pobaire et al. (1977).

9. Since the possibility of the seed extract affecting the hormone-receptor interaction at the target organs has been suggested, work in this direction also should be carried out.

10. Composition of fluid in the epididymis of control and treated rats by micropuncture and microanalysis techniques is called for.

11. Studies regarding any change in the contractile mechanism of epididymides after treatment should be investigated.

12. Any change in proteins which are unique to epididymis and which are coated as sperm specific antigens on spermatozoa during their passage through epididymis, their characterization and action has to be studied under control and treated conditions.
13. Studies involving osmotic balance of the luminal fluids, pH changes, the occurrence, concentration and the role of several metal ions especially Na, K, Ca and Mn have to be carried out under normal and seed extract treated conditions.

14. Ultrastructural make up of androgen target organs in treated animals need to be carried out.

15. Systematic toxicity studies using standard procedures for brain, thyroid, adrenal, liver, kidney and blood functions have to be carried out.

16. Finally success of the rat trials necessitates the retesting of the crude and pure material obtained from Carica papaya seeds by other laboratories in India or abroad in monkeys later on in human beings.

17. This would lead the way to induce functional sterility in males using a plant product which is hitherto not investigated and holds such promise.

11. FUTURE LINES OF WORK ON VINCIA ROSEA LEAVES

Although Vincia rosea is more well known for its anticascinogenic activity by virtue of its many indole alkaloids the antifertility effects of this plant can also be studied in detail by isolating the major principle which is responsible for the induction of functional sterility. However, thorough
investigation on the toxic effects of this plant product has to studied on various para-reproductive organs before any conclusive reversible antifertility effects could be made use of. For this purpose future work should be carried out in the same lines as stated for Carica papaya seed extract, which also might prove to be a successful male contraceptive.