The effects of short term (7 and 15 days) administration of aqueous extracts of *Carica papaya* seeds (1 mg/0.2 ml/day/rat) on the histophysiology of testis, caput and cauda epididymides, proximal and distal regions of vas deferens (VDP and VDII), seminal vesicles and other accessory sex organs as well as fertility rate were studied in adult male albino rats of proven fertility. Usu of aqueous seed extract was found to be more effective in comparison to the extracts in various other organic solvents, viz., alcohol, benzene, petroleum ether and chloroform (Chinoy et al., 1984 c). Stress was laid upon the investigations of epididymides and vas deferens as potential sites for post-
testicular male fertility regulation, since no structural and biochemical alterations were observed in the testis of treated rats. The parameters studied were the sperm count and percent motility of testis and cauda epididymis, fertility rate, sperm morphology, biochemical study on some androgen-dependent constituents of caput and cauda epididymides; two regions of vas deferens, caput and cauda epididymal sperm suspension as well as their luminal fluid, electro-physiological studies on the contractile pattern of isolated left and right vas deferens and hormonal levels (testosterone, FSH and LH in the sera of control and treated groups of animals.

The possible reversibility of the induced effects have also been investigated by one and 2-2½ months withdrawal of treatment. The effects of combined extract + ascorbic acid (AA) feeding to 7 and 15 day extract treated animals were also investigated, since it is known that ascorbic acid synergizes with the endogenous testosterone and potentiates its anabolic action in androgen target organs (Chinoy, 1978; 1984; Chinoy, et al., 1979 a; 1982 b; Chinoy and Chinoy, 1983 a).

The body weight of treated rats did not vary significantly throughout all the treatments nor the weights of epididymides and vas deferens. The biochemical data revealed that the extract manifested an androgen deprived effect to the target
organs as most of the androgen-sensitive parameters were altered after treatment to a variable extent therein. The histoarchitecture of caput and cauda epididymides and proximal and distal vas deferens also changed to a variable degree in treated animals. In the whole, the cauda epididymis and the distal vas deferens were affected more by the treatment than the caput epididymis and proximal vas deferens in agreement with the earlier observations from our laboratory. This might probably be due to their differential sensitivity to androgens for maintaining their structure and metabolic functions (Chinoy et al., 1973 a; Chinoy and Chinoy, 1983 a), since not only the histology of proximal and distal regions of rat vas deferens, but also their biochemical components manifest differential sensitivity to the same androgen as well as to weak and potent androgens. On the whole, the distal vas deferens exhibited a higher threshold requirement of testosterone for the maintenance of its structural and biochemical integrity in comparison to the proximal region. These observations support the present work. The changes observed included alterations in the activities of succinate dehydrogenase, acid phosphatase, seminal vesicle fructose, protein levels especially in the proximal vas deferens and decrease in the contractile response of vas deferens (left and right sides) by 7 and 15 days treatments. These changes were coupled with histological alterations especially in cauda
epididymis, distal vas deferens and in seminal vesicles which support the androgen deprived effect to the target organs. Similar results have been obtained by Chinoy and Saam (1984) and Chinoy et al. (1984 b,c), using Carica papaya seed extract on adult male albino rats.

In order to ascertain whether the androgen deprivation effect at the target sites was as a result of the probable reduction in androgen levels or otherwise, a group of rats were treated simultaneously with papaya seed extract + testosterone (Chinoy et al., 1984 b). In this group of rats, the androgen dependent parameters in testis and accessory sex glands were unaffected and their levels were comparable to those in control animals. This data reveals that testosterone levels might be affected or else there occurs an interference with the uptake of androgens by the target cells. However, the levels of serum testosterone, 3α and 17β, cholesterol concentrations in testis and epididymis as well as testicular 3 β and 17 β-hydroxy steroid dehydrogenase activities were not significantly altered in treated rats as compared to control, which suggests that the extract does not affect testicular steroidogenesis. The histology of testis (in both 7 and 15 days treated rats), tubular diameter, germinal epithelial cell height, the activity of testicular SDH and Leydig cell diameter were also not significantly affected by the treatment (Chinoy and Saam, 1984; Chinoy et al., 1984 b,c).
Similar results on testicular histology have also been reported by Das (1980). Therefore, the data suggests that neither testicular steroidogenesis nor pituitary gonadotropin levels were altered by the papaya seed extract treatment. Thus the possibility of the extract manifesting its effects via pituitary-gonadal axis is eliminated. Moreover, since the testicular sperm count was also unchanged, it is evident that spermatogenesis occurs unhindered. However, it may be mentioned here that 15 days is too short a period to have a clear picture about spermatogenesis in rat, but the data of ongoing studies in the laboratory corroborate our observations.

The papaya seed extract treatment caused reduction in the number and motility of cauda epididymal sperms and altered their morphology. The vas deferens sperms were also significantly affected. These effects subsequently resulted in the complete loss of fertility within 7 days of treatment. Similar results have been obtained in rats with a much lower dose (0.1 mg/0.2 ml/day/rat) by Chinoy and Sam (1974), who have also established the minimum effective dose for causing reversible infertility without grossly affecting the histo-architecture of the reproductive organs. However, Das (1980) reported about 40% loss of fertility of male rats without any change in the pattern of their sperm motility, nor in the mating behaviour after papaya seed treatment. His results were obtained by administering 20 mg/kg body weight seed
powder orally/rat/day, whereas, a dose of 1 mg/0.2 ml/day/rat (5 mg/kg body weight) was injected intramuscularly in the present study.

It might be mentioned here that the fertility rate of female rats treated with the same dosage of aqueous papaya seed extract was also zero percent by 7 days treatment (Chinoy and Trivedi, 1980, 1983; Trivedi, 1982). In female rats and mice, the antifertility effects of papaya seed extract was mainly due to its anti-implanation action (Garg et al., 1970; Sareen et al., 1961) and abortifacient effects (Chinoy and Trivedi, 1980; 1983; Trivedi, 1982).

Alterations in the metabolism of rat cauda epididymal spermatozoa coupled with a reduction in their motility have been reported elsewhere (Chinoy et al., 1984 d). These workers observed a fall in motility concomitant with a decrease in protein content of the spermatozoa after treatment with papaya seed extract at a dosage of 0.1 mg/0.1 ml/day/rat for 7 days intramuscularly. A decrease in protein might affect the glycoprotein contents of the spermatozoa. Evidence from the work of Lee et al. (1978) suggests that glycoproteins secreted by the epididymis are coated on the surface of spermatozoa and an epididymal glycoprotein is capable of stimulating their motility (a protein which helps in their forward motility) in bull spermatozoa (Brandt et al., 1978).

In the present study too, protein levels declined in caput
and cauda epididymides and VDP and VDD tissues of 15 day treated rats and in sperm suspensions of caput and cauda epididymides after 7 days treatment. It is evident that a decrease in specific proteins might be related to reduction in sperm motility. It is likely, that the reduction in ATPase activity in spermatozoa of treated animals (Chinoy et al., 1984 d) might be one of the contributory factors towards the decrease in sperm motility, since it is known that this enzyme plays an important role in the energy metabolism of spermatozoa. The decrease in ATPase activity might inturn be due to changes in the metal ion concentrations (Ca, Mg or Na) which are responsible for its activation. The spermatozoan flagellar axoneme contains the contractile protein domain which is rich in ATPase and is supposed to play an important role in converting ATP into mechanical energy needed for flagellar movement (Gibbons, 1977). The decline in succinate dehydrogenase activity of sperm suspension of caput and cauda epididymides, in the present study support the above observations in that the oxidative/energy metabolism of spermatozoa is altered by treatment.

This is further corroborated by the recent observations (Chinoy et al., 1984 c) that the in vitro motility of caput, cauda and vas deferens sperms were reduced significantly by 40 minutes in a medium containing HEPES buffer and the aqueous seed extract in equal volumes. The motility was however, zero
per cent by 60 minutes. The effect was cauda > vas deferens. On the contrary, the treatment did not alter the levels of glucose, glucose - 6 - phosphatase or fructose- 1,6-diphosphatase activities significantly, indicating that the glycolytic metabolism of sperms was not changed except for a decline in phosphorylase activity. These workers (Chinoy et al., 1984 d) have also reported a decrease in the acid phosphatase activity in spermatosoa of treated rats in accordance with our present work, where the enzyme activity declined in the epididymides and vas deferens as well as in the sperm pellet of caput epididymis of treated rats. This is probably correlated with the resorption of dead and non-motile spermatosoa which might account for the decrease in epididymal sperm density in treated rats obtained in the present study.

A detailed study on the microenvironment of the epididymides is being carried out in control and treated rats by using the micropuncture technique (Chinoy et al., 1983 b) which will probably provide a clearer insight into the alterations in sperm metabolism after treatment. These studies are being coupled with ultrastructural observations on the spermatosoa.

The treatment brought about significant changes in the morphology of cauda and vas deferens sperms as observed by Scanning Electron Microscopic (SEM) studies. The changes
observed included decapitation of several spermatids with damage to the acrosomal region, as compared to the control sperms. It is well known that acrosome plays a major role in the fertilization of spermatids since it contains several lytic enzymes (acrosin, β-glucuronidase, hyaluronidase, acid phosphatase and alkaline phosphatase), and any damage to the acrosomal region might result in the loss of these enzymes, rendering the sperms non-viable. Hence, it is suggested that the alterations in sperm morphology in treated rats might also be responsible for the reduction in fertility rate.

The present data elucidates that the androgen deprived condition especially of cauda epididymis led to alterations in its structure and metabolism and probably its micro-environment, thereby rendering its milieu hostile for sperm maturation and viability, which in turn affected the sperm morphology, their metabolism, motility and eventually lowered the fertility rate in treated rats, since it is known that the abnormal composition of the epididymal plasma affects sperm morphology and metabolism and reflects on a functional disturbance in the epididymal epithelium (Gustafsson et al., 1974).

The electrophysiological experiments carried out to study the contractile response of isolated left and right vas deferens to different doses of adrenaline suggested a dose
dependent change in the contractile pattern in both control and treated animals. However, in 7 day treated animals there occurred a significant reduction in the contractile response of both left and right vas deferens. This might be due to the increased muscle layer thickness and other alterations in the histology as a result of the treatment (Chinoy and Geetha Ranga, 1984). These results were similar to those obtained under several other treatments (Chinoy and Chinoy, 1979; 1981; 1983 b). It is likely that the reduced contractile response of vas deferens in treated rats might also be a contributing factor in reducing fertility in treated rats, since it is known that vas deferens is not merely a conduit for sperm transport but it contributes to the sperm metabolism, viability and survival under normal physiological conditions (Chinoy and Chinoy, 1982 a; 1983 a; Chinoy et al., 1983 g).

All throughout the studies the contractile response of isolated left vas deferens was much more than that of the right one to different doses of adrenaline in accordance with earlier observations from our laboratory (Chinoy and Chinoy, 1983 b). This differential response of the two sides of vas deferens from the same animal to increasing doses of adrenaline has been correlated with the higher concentrations of calcium and sodium in the right vas deferens, since it is known that the high levels of membrane-bound calcium
renders the cell less excitable. It was suggested (Chinoy and Chinoy, 1983 b) that the difference in response of the right and left sides of vas deferens might also be due to the levels of intracellular calcium, which is bound to calmodulin, a polypeptide (Cheung, 1960; Slater, 1981), which acts as an activator for norepinephrine release and muscle contraction as well as other cellular processes, only when it is bound to calcium. It was therefore, presumed by Chinoy and Chinoy (1983 b) that the left vas deferens might possess more calmodulin bound calcium than the right vas, which has instead, more membrane-bound calcium. The results of the present study support these observations. Hence, it is suggested that further studies are required to investigate the levels of bound and free forms of calcium in the vas deferens of the two sides under control and physiologically altered conditions, since it plays an important role in the release of neurotransmitters and muscle contraction via activation of calmodulin, the calcium-binding protein (Lenz and Corriner, 1982). This calmodulin-bound calcium acting synergistically or antagonistically with c-AMP (Means et al., 1982) is involved in discrete cellular activities as muscle contractions, sperm motility and neurotransmitter release etc (Slater, 1981). A thorough understanding of the fine structure of smooth muscle of vas deferens, determinations of the levels of free and bound calcium and calmodulin would
help to understand the mechanism, whereby, contractile pattern is modulated. The use of specific Ca\(^{2+}\) inhibitors or calcmodulin-binding drugs among then might be advantageous in the development of a male contraceptive.

That the extract had no serotonin or 5a-5HT-like activity was checked by carrying out an experiment in which the control isolated vas deferens was immersed in extract solution and its contractions were recorded. However, the vas deferens did not respond at all to varying doses. Therefore, the view of Farnsworth (1975) that the active principle responsible for anti-implantation effect of Carya ovata seed in female rats might be 5α-hydroxy tryptamine or serotonin-like activity is not true in the case of male rats as revealed in the present studies.

In both 7 and 15 day seed extract treated animals, an enhanced ascorbic acid metabolism was observed in caput and cauda epididymides and proximal and distal regions of vas deferens. This resulted in an increase in the conversion of bound form of ascorbic acid to its free form and concomitantly a much higher rate of utilization under treated conditions. However, the cholesterol levels of testis and epididymides were not affected. These changes are in agreement with earlier observations from our laboratory that ascorbic acid turnover pattern is significantly altered in several drug-treated conditions indicating its importance in the
metabolic functions of reproductive tissues (Chinoy, 1978; 1984; Chinoy et al., 1982 b).

In one group of rats the seed extract was administered and thereafter the treatment was withdrawn for a period of 1 to 2.5 months, in order to investigate the possible reversibility of the induced effects. The data revealed that the organ weights, sperm counts, percent motility and morphology of cauda epididymal sperms, rate of fertility, almost all androgen dependent parameters recovered to a significant extent in comparison to the extract treated rats. A similar recovery was observed in the histology of all male reproductive organs (caput and cauda epididymides, proximal and distal regions of vas deferens and seminal vesicles and the contractile response of vas deferens of treated rats after one and 2.5 months discontinuation of treatments. Similar results have been obtained for the histophysicsiology of accessory reproductive organs after three months withdrawal of treatment with a lower dose (0.1 mg/0.2 ml/day/rat) (Chinoy and Ham, 1984), which suggested that the effects of the extract were transient and reversible.

In rats which were given extract treatment for 7 and 15 days and were fed simultaneously with ascorbic acid (AA) (50 to 100 mg/day/rat) the recovery in almost all the androgenic parameters was similar to, if not more, in comparison to withdrawal treatment. In most of the organs
studied, an enhanced ascorbic acid metabolism occurred in treated rats with an increased mobilization of bound ascorbic acid to its free form and concomitantly a much higher rate of its utilization. This elucidated that ascorbic acid helps in overcoming the effects of the extract, but does not interfere with the contraceptive action. The above data substantiates the hypothesis that increased ascorbic acid utilization has a prophylactic influence on the metabolism of the testis and accessory sex glands in drug treated rats in corroboration with earlier findings from our laboratory (Chinoy, 1976; 1982a; Chinoy et al., 1982b).

All the above data elucidates for the first time that the androgen deprivation and antifertility effects induced by Carica papaya seed extract treatment were transient and reversible by withdrawal of treatment as almost complete recovery in fertility rate and histophysiology of the reproductive organs could be achieved in corroboration with other reports from our laboratory.

That papaya seed extract is a potential male contraceptive agent, of reversible nature is an important observation and further toxicity trials and related investigations are being carried out, since it seems to have little or negligible side effects. Its reversible antifertility effects in females have also been reported (Chinoy and Trivedi, 1980; 1983).
Das et al. (1980) suggested that ripe papaya seed powder might have some toxic effect as demonstrated by the reduction in body weight gain with age in male rats. On the contrary, the present study indicated that the body weight was not affected and there occurred no mortality of treated animals. That the extract manifests no toxic effects is evident since serum SGPT, SGOT, cholesterol and protein levels were within the normal limits in the extract treated animals (Chinoy et al., 1980). Further studies are underway in our laboratory to elucidate any effects of the papaya seed extracts on para-reproductive organs such as brain, thyroid, liver, kidney, adrenal and blood.

As has been reported in the earlier part of the discussion the extract did not manifest any anti-agonadotropic effect, nor reduced the serum testosterone levels and yet the androgen-sensitive metabolism of target organs, especially that of the epididymides and vas deferens was altered to a variable extent. It is therefore likely that this androgen-deprived effect might be caused by low target organ response and/or the reduced conversion of testosterone to its potent metabolites. It would hence be worthwhile to study the levels of 5α-3DH, Δ⁴-5α-reductase and 3α-hydroxy steroid dehydrogenase activities in the epididymides of control and treated animals according to the method of Vobaire et al. (1977). The 3α-hydroxy steroid
dehydrogenase (3α-HSD) is an androgen dependent enzyme in caput and cauda epididymides, whereas, the major factor regulating Δ⁴-5α-reductase activity, is a substance directly secreted into the epididymis by the testis, probably ABP (Robaire, 1978; Robaire et al., 1981) and that the activity of this enzyme is altered under experimental conditions (Robaire and Zirkin, 1981). It is likely that the extract treatment might also alter the hormone-receptor interaction at the target cells and thereby, interfere with the hormone action. Detailed studies in this direction are being undertaken.

In conclusion, it is clear that Carica papaya seed extract (aqueous) has definite antifertility effects which were reversible. Thus functional sterility could be induced. This treatment has promise as a potential post-testicular male contraceptive with apparently little side effects.
PART II

1. STUDIES ON THE EFFECTS OF AQUEOUS EXTRACTS OF VINCA ROSEA LEAVES ON ADULT MALE ALBINO RATS FOR 7 AND 15 DAYS.

2. CASTRATION + TESTOSTERONE TREATMENT FOR 7 DAYS.

CASTRATION + TESTOSTERONE + EXTRACT TREATMENT FOR 7 DAYS.

3. EXTRACT TREATMENT + ASCORBIC ACID FEEDING FOR 7 AND 15 DAYS.

4. EXTRACT TREATMENT FOR 7 DAYS + DISCONTINUATION OF TREATMENT FOR 1 AND 2 MONTHS RESPECTIVELY.

Studies on the male reproductive physiology after short term administration of aqueous Vinca rosea leaf extracts (1 mg/0.2 ml/day/rat) revealed antiandrogenic and antifertility effects in intact adult male albino rats. The reproductive organs studied included testis, caput and cauda epididymides, proximal and distal regions of vas deferens (VDP and VDI) and seminal vesicles. As in the case of Carica papaya seed extract treatment, emphasis was laid upon studying the physiology of epididymides and vas deferens besides other accessory sex organs. The parameters studied were body and organ weights, count and percent motility of cauda epididymal spermatozoa, fertility rate, sperm morphology (by
Sil studies), and circulating levels of serum testosterone and gonadotrophins (FSH and LH) in control and 7 and 15 day leaf extract treated rats. The antiandrogenic effect of the extract was studied by comparing certain androgen sensitive parameters of castrated + testosterone treated and castration + testosterone + leaf extract treated rats.

The beneficial effects of ascorbic acid feeding to 7 and 15 day extract treated animals were also studied. The recovery of the androgenic parameters was investigated in extract treated rats where the treatment was discontinued for a period of 1 and 2½ months respectively.

The data revealed that the leaf extract treatment manifested antiandrogenic and antifertility effects in intact male albino rats. The antifertility effects were attributed to reduction in sperm density and percent motility of cauda epididymal sperms as well as alterations in the morphology of cauda epididymal spermatozoa after treatment for 7 and 15 days. The sperms were sluggishly motile in Vincra rosea leaf extract treated males and were unable to fertilize normally cycling females both under 7 and 15 day treated conditions, thus leading to zero percent fertility rate.

The Vincra rosea leaf extract treatment manifested a strong antiandrogenic effect thereby causing reduction of most of the androgenic parameters in androgen target organs of treated animals, viz., a significant decrease (15 days
treatment) in their body weights, organ weights of pituitary, testis, caput and cauda epididymides, VJP and VJD and seminal vesicle fructose levels. The enzyme succinate dehydrogenase (SDH) is a mitochondrial enzyme, known to be a useful index for evaluation of sperm density and epididymal functions in mammals including human beings (Prasad et al., 1972; Khatoon, 1973), as it is an androgen sensitive enzyme. This enzyme is contributed by accessory sex glands (Feth and Rao, 1959; Mann and Lutwak-Hanger, 1981). Acid phosphatase is a lysosomal enzyme and it is an indicator of prostatic function in mammals. Our results of 7 day treated animals indicated that acid phosphatase activity decreased significantly in caput and cauda epididymides along with a reduction in sperm density. However in 15 day treated animals the activity showed marked increase, which might be due to the lytic activity taking place in the epididymides as well as in VJP and VJD due to damage and degeneration of spermatozoa.

The accumulation of cholesterol in caput and cauda epididymides and also in the distal vas deferens of 7 day treated animals is an indication of reduced steroidogenesis in the testis in the initial stages of the treatment as compared to control tissues. However, the levels of circulating serum testosterone did not show any significant variation from the normal. However, as mentioned earlier, the extract manifested strong androgen-deprived effects to target tissues,
which was further substantiated by the results of treatments imparted to groups IV and V rats, i.e., castrated + testosterone treated and castration + testosterone + leaf extract treatment. A significant decrease in the seminal fructose levels with a concurrent decrease in its glandular epithelia and secretions (present study) as well as those of the ventral prostate (VP; Bina Jacob, 1982) support the androgen deprived condition of the target organs in treated animals. The antianrogenic effect on caput and cauda epididymides of 7 and 15 day leaf extract treated rats have already been reported elsewhere (Chinoy and Geetha Fanga, 1983).

The decrease in the total ascorbic acid (TAA) and glutathione levels in both caput and cauda epididymides and proximal and distal regions of vas deferens (VDP and VDD), coupled with a significant accumulation of cholesterol in testis (Bina Jacob, 1982) of treated rats suggests reduced testicular steroidogenesis in such animals, since it is known that ascorbic acid is involved in steroidogenesis in the testis (Datta and Sanyal, 1978; Chinoy et al., 1982 b), as well as hydroxylation and oxidation of cholesterol (Chinoy, 1978). That the Vinca rosea leaf extract treatment affects testicular hormoneogenesis is further substantiated by the fact that in castrated rats treated with plant extract + testosterone (group V animals), the androgen sensitive
parameters were not maintained as compared to group I animals, except protein in caput and SDH in the cauda. In another group of intact rats which were given plant extract + testosterone injections for 7 days, the androgenic parameters were however unaffected and their levels were comparable to those in the control rats (Bina Jacob, 1962).

In all the four tissues of treated animals, a very significant increase in the free ascorbic acid levels and a decline in ascorbigen occurred which was an indication for the rapid mobilization of bound ascorbate and its enhanced utilization. All reproductive organs as well as spermatozoa are able to utilize ascorbic acid, since they possess the necessary enzymes for its metabolism. The rate of utilization of ascorbic acid increases under any imposed stress conditions and involves the mobilization of bound form of ascorbic acid to its free form (Chinoy et al., 1982 b). The utilization of AA occurs via the formation of its free radical, monodehydroascorbic acid (MDHA), which is a more powerful reducing agent than ascorbate by virtue of possessing an unpaired electron. The second pathway of utilization is via formation of ascorbate macromolecule charge transfer complexes (Chinoy et al., 1978 b). It is involved in enzyme activation, electron transport, metabolism of proteins, nucleic acids, carbohydrates, lipids, minerals as well as in those of brain and muscles (Chinoy et al., 1982 b).
Ascorbic acid has an important relationship with several hormones including testosterone, epinephrine and norepinephrine (Levin, 1976; Chinoy and Hao, 1979; Chinoy et al., 1979 b). It inhibits the activity of phosphodiesterase (Levin, 1976) and thereby, indirectly helps in increasing the levels of cAMP, which is an important "second messenger" in mechanism of hormone action.

Histocytometric studies on caput and cauda epididymides and VDP and VDD tissues also substantiated earlier biochemical observations. However, 15 day's treatment brought about much more changes to all the four tissues including testis than 7 days treatment. The changes observed might be due to androgen deprived effects to target tissues. Of all the tissues studied, cauda epididymis and proximal vas deferens appeared to be affected more by the treatment than the other tissues indicating higher androgen requirement of these tissues for maintenance of their structure and functions in accordance with earlier observations from this laboratory (Chinoy and Chinoy, 1983 a).

Scanning electron micrographic study of treated rat cauda epididymal spermatozoa revealed that the majority of sperms were decapitated due to treatment.

However, no significant change in the levels of testosterone, FSH and LH in the treated rats were observed except in one month withdrawal group wherein, a significant
increase in testosterone (T) levels and decrease in FSH levels occurred as compared to control animals. This is an important manifestation since it suggests that the leaf extract does not bring about its antiandrogenic effects through disturbances of pituitary-gonadal axis at least initially during the treatment. Later there occurs a latent effect so that decrease in FSH but increase in testosterone and reduction in pituitary weight resulted. That the extract manifests a long lasting effect on pituitary-gonadal axis is also evident from the decrease in the testis weight and changes in its histology especially by 15 days treatment.

Thus it is suggested that *Vinca rosea* leaf extract treatment exerts antifertility effects in a short time interval since, it reduces the epididymal spermatozoal motility, causes alterations in their morphology, in the biochemical profile and histomorphotecture of all the organs studied, which led to zero percent fertility rate within 7 days of treatment. Similarly the fertility rate of female rats treated with aqueous *Vinca rosea* leaf extract was also curtailed (Chinoy and Trivedi, 1980; 1983; Trivedi, 1982). Thus it is evident that the antiandrogenic effects of the extract are coupled with its antifertility effects. However, the present studies as well as those by Bina Jacob (1982) revealed that the accessory gland structure and metabolism seemed to be affected more than that of the testis.
In order to study the reversibility of the induced effects of the plant extract to two groups of rats the treatment was imparted for 7 days as in groups II and thereafter it was ceased for 1 and 2½ months respectively. Most of the androgen sensitive parameters including organ weights, recovered significantly in almost all the organs, viz., caput and cauda epididymides and proximal and distal vas deferens by one month withdrawal period and recovery in some parameters was much beyond control levels by 2-2½ months withdrawal.

A recovery in the histomorphology of epididymides and vas deferens and in ascorbic acid turnover was also observed after one and 2½ months withdrawal. Similar observations were made on the recovery of the histology of testis and accessory organs (Bina Jacob, 1982). However, the recovery in fertility rate was only 45% even after 2 to 2½ months cessation of treatment indicating that a longer period is required for the complete recovery of fertility rate.

In order to study the beneficial effects of ascorbic acid under physiologically altered conditions, groups VI and VII rats were given *Vincetoxicum hirundinaria* leaf extract treatment and were simultaneously fed with 50-100 mg of ascorbic acid orally. The results revealed that extraneous ascorbic acid feeding helped in restoring some of the androgen sensitive parameters almost to normal levels. The histology of the reproductive tissues was also restored significantly after ascorbate
feeding. This substantiates the fact that oral feeding of ascorbate resulted in beneficial alterations in the reproductive tissue metabolism as discussed by Chinoy (1978a, 1984; Chinoy et al., 1982b) in various other experimental conditions.

Deficiency of Vitamin C has been known to cause atrophy of the testis (Chatterjee, 1967), impaired androgen synthesis (Bhalavady and Jasanjee, 1954), caused spermatogenic arrest (Mishra, 1967) decreased sperm motility, accumulation of lipids in testis epididymis and vas deferens (Chinoy et al., 1982b) altered the sperm morphology and raduced the fertility rate in scorbutic guinea pigs (Chinoy et al., 1984a). Biochemical and 3T data on the ascorbate metabolism of semen of normo, oligo and azoospermic human male volunteers suggested for the first time, that AA has an important role in sperm motility and metabolism via the formation of MDHA (Chinoy and Buch, 1977b).

The above discussion indicates that by Linca Rosa leaf extract treatment results in functional sterility in male rats. By monitoring of the proper dose regimen, a longer withdrawal period and thorough investigation of the side effects, it will be possible to pave the way for the development of a simple, easy to use contraceptive for males, whose effects are reversible. The data on the group of rats treated with the extract for 7 and 15 days and fed with
ascorbic acid simultaneously, indicates that such a treatment was more effective for recovery of majority of the androgenic parameters and structure of most of the androgen target organs, than the discontinuation of treatment. This again proves that ascorbic acid helps in overcoming the induced anti-anabolic effects of the extract. The beneficial role of ascorbic acid and its mechanism of action in male contraception have been highlighted previously.

The mechanism of action of *Vinca rosea* leaf extract appears to be directly on the testis and epididymides. However, a possibility that the extract might affect the functions of the pituitary-gonadal axis also cannot be ruled out especially in 15 day leaf extract treated rats. An effect on testicular steroidogenesis either directly or indirectly is also likely to occur. That the extract affects the neuroendocrine mechanisms is supported by the fact that mammalian nervous tissue and their subcellular components are capable of uptake of *Vinca alkaloids* (Zafer Iqbal and Ochs, 1980).

Although the body weight of the treated animals did not alter much except in 15 day treated animals, the extract did not seem to possess long standing severe toxic effects. Nevertheless, proper systematic studies on toxicity in the treated animals as well as effects of the extract on the brain, thyroid, liver, kidney, adrenal and blood functions need
to be carried out inorder to ascertain the toxic nature of
the extract, if any. However, the liver and kidney histology
were not altered by leaf extract treatment in rats (Chinoy
and Geetha Langa, Unpublished observations).

From the above discussion on the effects of the two
plant extracts on male fertility regulation, it is clear
that the aqueous extracts of Carica papaya seeds as well as

Vinca rosea leaves could induce reversible antifertility
effects in adult male albino rats of proven fertility.
However, the data permits the conclusion that papaya seed
extract has several obvious advantages over Vinca rosea
leaves extract due to the following reasons.

At no stage during the treatment with papaya seed
extract a reduction in the body weight could be discerned,
which however, was not the case especially in 15 day Vinca
rosea leaf extract treated rats. The antiandrogenic effects
of the Vinca rosea leaf extract are coupled with its anti
anabolic action, as is evident from the decrease in body
weights, those of the reproductive organs as well as of
pituitary. No such changes were observed after papaya seed
extract treatment. Although some histological alterations
in the reproductive organs were observed by both the treat-
ments, the severity was more by Vinca rosea treatment than
by the papaya seed. Besides, the testicular histology was
affected by the leaf extract treatment only along with an
increase in its cholesterol concentrations indicating a probable reduction in steroidogenesis; which however, was not manifested in papaya seed extract treated animals.

The changes in the biochemical profile, and histoarchitecture of all the reproductive organs studied were although transient and reversible by 1-2 months discontinuation of treatment in *Vinca rosea* leaf extract treated animals; it is clear that it might require a much longer period and/or a lesser dosage as compared to papaya seed extract treatment, since the recovery obtained was less significant in the former case.

The fact that the leaf extract did not bring about any change in the serum testosterone or FSH and LH levels initially, but showed some variation in withdrawal group of rats indicates that the leaf extract exerts a slow and late acting influence on the pituitary-gonadal axis. However, in papaya seed extract treated animals the pituitary gonadotrophin and testosterone levels did not show any significant variation either after treatment or during the withdrawal period.

It is also a well established fact that Vinca alkaloids are anti-mitogenic in action and can also affect the neuro-endocrine mechanism under various conditions. However, no toxic effects of papaya seeds have so far been reported so far, although the adulteration of dried pepper seeds with dried
Papaya seeds has been done. Taking into consideration all the above facts it is concluded that papaya seed extract can be a more feasible approach for male fertility regulation in manuals than Vincas rosea leaf extract treatment. Further studies in this direction are underway.