SUMMARY AND CONCLUDING REMARKS
In spite of the availability of large number of contraceptive methods like the mechanical devices, bioactive devices, surgical procedures and drugs for the control of fertility, search still continues for the development of newer, safer, easier and more effective methods to combat the population explosion. The entire area of male physiology of reproduction has been generally ignored, the main emphasis being given to the interference in the female physiology of reproduction. But most of the bioactive devices and hormonal preparations have been shown to exert side effects. Therefore in recent years extensive efforts are being made to find out an effective possibly non-hormonal preparation from plant sources with fewer or no undesirable side effects for control of human fertility. Such widespread interest in the evaluation of plants for fertility control in male prompted the undertaking of the present studies on the antispermaticogenic potentials of Butea monosperma leaf extracts.

The review of the work on plant extract induced aspermato­genesis shows that most of the workers have focussed their attention only on histological alterations in testes. Such a study of histological changes in the testes brought about by the antispermaticogenic agents gives only a partial information on induced aspermato­genesis, hence a study of other biochemical and enzymatic parameters is also highly desired. Moreover in such induced aspermato­genesis, study of histological and metabolic alterations in testes alone is not suffi­cient, since such antispermaticogenic agents may also exert some influence, either directly or indirectly, on accessory male sex organs. Hence a study of histological, metabolic and enzymatic alterations in accessory sex organs is also essential to understand
the action of antispermatic agents. Keeping these points in view in the present investigation the effects of *Butea monosperma* leaf extract administration have been studied both on testes and accessory organs of white rats. Thus the present investigation has been carried out with reference to alterations induced by *Butea monosperma* leaf extract administration in wet weights of organs, histoarchitecture, three lysosomal and one nonlysosomal enzymes, mucosubstances, lipids and proteins in testes and major sex accessory organs of the male reproductive tract of albino rats.

The *Butea monosperma* water soluble alcoholic leaf extractive was administered intraperitonially to rats of 100 days age and 275 gm body weight over a period of 90 days. The histological alterations were studied by routine haematoxyline-eosin and PAS-haematoxyline techniques supported by Feulgen technique. Studies on changes in lysosomal enzymes *viz.* β-glucuronidase, acid phosphatase and nonspecific esterase, and a nonlysosomal enzyme alkaline phosphatase were carried out by employing both bioassay and histochemical techniques. Distribution and alterations in the mucopolysaccharides were investigated by using a battery of recent and well developed histochemical techniques. The lipids comprising of total lipids, neutral lipids and phospholipids were studied by thin layer chromatographic and bioassay techniques. The total proteins were investigated by bioassay method. To make the present investigation technologically as perfect as possible both histochemical and bioassay techniques have been employed.

The histological, enzymatic and other metabolic investigations were carried out at an interval of 15 days over a period of 90 days.
I. Testis:

1) Alterations in wet weight and histology:

_Butea monosperma_ leaf extract administration to albino rats resulted in 25% decrease in wet weight of the testes. An age related increase in the body weight was evident in control rats, but not in the extract treated rats. The important feature of the present investigation was that all spermatogenic cell elements with the exception of spermatogonia were affected due to the extract treatment. The effects of the extract ranged from mild damage to germinal elements to their total destruction depending on the duration of the treatment. The spermatogenesis appeared to be arrested mainly at spermatid stage. Comparatively more damage was observed in spermatids than in spermatocytes. Formation of clear spaces and vacuoles in and inbetween tubules and in the cells was also observed. Chromatolysis was also evident in the secondary spermatocytes. The debris was evident in lumina of the seminiferous tubules and was seen as a plug. It contained spermatogenic elements such as spermatocytes, spermatids etc. which were sloughed off from the degenerating tubules. Due to vacuolization in Sertoli cell, the sloughing off of premature spermatozoa was also observed, but this was rather a rare phenomenon. Due to severe deformities in basement membrane, some tubules were much swollen, some were shrunken and very few showed pseudopodia like projections. The _Butea_ leaf extract exerted very poor effect on Leydig cells, which did not show any significant histological alterations. The presence of both severely damaged and normal seminiferous tubules in the same sections of testis, especially in last phase of treatment, was also evident. The active principle seems to affect the structural
or chemical constituents playing important role in meiosis. The Butea leaf extract seems to interfere with spermatogenic cells directly.

2) **Lysosomal enzymes**

The three lysosomal enzyme, β-glucuronidase, acid phosphatase and nonspecific esterase showed enhancement in the first 15 days of Butea leaf extract treatment. Then with insignificant decrease or increase up to 60 days of treatment, the activities of all the enzymes were much enhanced as compared to the control, when the seminiferous tubules were in very active stage of degeneration in the final phase of the extract treatment.

The enzymorphology of lysosomal enzymes in different cellular elements of testes with different histochemical staining techniques exhibited some noticeable alterations. β-glucuronidase was localized in spermatogonia, spermatocytes and round and long spermatids; acid phosphatase in spermatocytes and long spermatids and in sperm heads; while esterase was localized in spermatogonia and long spermatids only. In the last phase of the extract treatment when cellular necrobiosis was advanced in affected cell types, the staining intensities of all lysosomal enzymes in such affected cell elements were very much increased. The debris in the lumina of seminiferous tubules showed intense activity, when cellular damage was more due to Butea leaf extract treatment. Involvement of the lysosomes and their acid hydrolases in the testicular damages caused by the Butea leaf extract was evident from these observations.

3) **Alkaline phosphatase**

The biochemical and histochemical studies of the behaviour of alkaline phosphatase showed practically identical pattern of
alterations as observed in the three lysosomal enzymes investigated in the present work. It was observed that at the initiation and termination of the extract administration, the enzyme activity was enhanced. The increase in both acid and alkaline phosphatases observed in the present investigation was a characteristic feature. One is a lysosomal enzyme and the other is a nonlysosomal one.

4) Mucopolysaccharides:

The histochemical distribution of different mucopolysaccharides observed in control rat testis showed the presence of glycogen and sialic acid in various quantities in all cell types of testis. Sertoli cells were devoid of glycogen, but contained neutral mucopolysaccharides. Leydig cells contained traces of glycogen, sialic acid and neutral polysaccharides. The Butea leaf extract induced reduction in glycogen and sialic acid contents of spermatogonia, spermatocytes and spermatozoa, reduction in sialic acid and increase in neutral mucopolysaccharides in Sertoli cells, and reduction of glycogen and absence of sialic acid in Leydig cells. The diagnostic feature in the present investigation was that nearly all types of mucosubstances were increased in the basement membrane.

5) Lipids:

The testicular lipid alterations involved: (1) increase in the total lipids, neutral lipids and their components such as glycerides and cholesterol, (2) decrease in the total phospholipids and their components like phosphatidyl choline and phosphatidyl ethyleneamine. The remaining components of neutral and phospholipids were not affected much.

The increase observed in total lipids, cholesterol and glycerides might be due to their nonutilization. Such an increase
might also be due to fatty degeneration of spermatogenic elements caused by Butea leaf extract. The depletion in phospholipids was mainly because of destruction of cellular membranes of which the phospholipids form a major component.

6) **Proteins**

Biochemically the total proteins in testes of treated rats showed a depletion. This might be due to depleted number of spermatozoa and other cellular elements present in practically empty tubules in the last phase of the extract treatment.

II. **Epididymis**

1) **Alterations in wet weight and histology**

The observations on caput and cauda epididymal parts showed a duration dependent depletion in the wet weight, which seemed to be due to less number of normal spermatozoa entering into the lumen of epididymis as a result of aspermatogenic action of Butea monosperma leaf extract. The histological alterations differed in the two parts of the epididymis. The epithelium of caput epididymis did not show any prominent changes in its structure. But the luminal contents, interstitium and basal lamina were affected. The luminal debris showed clumped spermatozoa, degenerating spermatozoa and immature germ cells like spermatids, spermatocytes and cytoplasmic masses without nuclei and giant cells. The cellular elements in the debris appeared to be derived from the damaged testes. The debris with its different cells was more after 45th day of the extract treatment, and then it got reduced after 60th day. This was possibly because of their passage out of epididymis or their phagocytotic removal.

In cauda epididymis the epithelium and interstitium were not affected, but the basal lamina was thickened. Due to total absence
of spermatozoa at the end of experiment, the lumina of few tubules appeared wide and big in size. The cellular debris was seen in very few tubules, but the identification of different cellular elements was not possible, may be because of changes in them in this part of epididymis.

2) Lysosomal enzymes:
The three lysosomal enzymes studied biochemically in the present investigation showed enhanced levels of their activities at the termination of experiment. The enhancement in nonspecific esterase was not significant, but in the other two enzymes it was quite significant. The alterations in epididymal β-glucuronidase and acid phosphatase in extract treated rats were not due to any androgenic imbalance, but appeared to be due to degenerative changes occurring in the lumina of the epididymal tubules and epithelial cells as seen from the histochemical observations in these enzymes. The number of intensely stained granules was increased in epithelial cells of caput and cauda epididymis in second phase of the extract administration.

3) Alkaline phosphatase:
Alkaline phosphatase activity was found to be increased in both the parts of epididymis when studied biochemically. Histochemically also the enzyme activity was enhanced in the epithelium of caput and cauda epididymis. It was interesting to note that the response of epididymal lysosomal enzymes and alkaline phosphatase, a nonlysosomal enzyme, was practically identical. The increase in acid and alkaline phosphatases suggested the toxic effects of extracts probably on interstitium and in lumen but not on epithelial cells, as they were not much altered. Such increase in acid and alkaline phosphatases in
epididymis might be helping in quick disposal of number of abnormal sperms formed in treated rat testes.

4) **Mucopolysaccharides**:

Glycogen, sialic acid and other mucosubstances were found to be decreased after the administration of *Butea* leaf extract. As suggested by many workers an optimum level of sialic acid is necessary for maturation and survival of spermatozoa in epididymis. Hence the reduced sialic acid contents in epididymis of treated rats may be affecting survival and maturity of whatever normal spermatozoa that happen to come to epididymis, thus, further reducing the fertility of treated rats.

5) **Lipids**:

In the extract treated rats, total lipids, neutral lipids and components of neutral lipids increased, while phospholipids and their components decreased in caput and cauda epididymis. It appeared that when normal sperms ceased to come to the epididymis and when their number and cellular debris got reduced in the epididymis, the total and neutral lipids exhibited an enhancement. Since the present studies were carried out biochemically, there was no way of knowing where exactly these lipid alterations might be taking place, whether in the epididymal epithelial cells or in the lumina of tubules. It appeared that total lipids, neutral lipids and their components accumulated due to their nonutilization, because of depletion in number of normal spermatozoa in the a spermatogenic condition induced by the *Butea* extract. As number of normal spermatozoa in epididymis of treated rats was reduced, the total phospholipids also might be getting reduced.
6) **Proteins** :

Total proteins of epididymis got depleted at the termination of treatment of *Butea* leaf extract. The protein alterations, when compared to other metabolites, were very inconsistent. The decrease in total proteins of caput was negligible, when compared with cauda epididymis.

**III. Seminal vesicles** :

1) **Alterations in wet weight and histology** :

Wet weight of seminal vesicles increased and this seemed to be due to accumulation of secretion in the lumina. The mucosal folds of seminal vesicles, which were highly arborized and reached up to the center of lumen in control rats, got reduced in height and arborization. The epithelial cells lining the mucosa showed slight reduction in height, which was observed conspicuously in later phases of the extract treatment. The muscular coat of the vesicles was thickened, many lumina were full of secretion, but few of them were devoid of secretion. These histological alterations appeared not to be due to altered androgenic state of the treated animals, but might be due to the direct action of the extract on the seminal vesicular structures.

2) **Lysosomal enzymes** :

The biochemical investigations of lysosomal enzymes showed enhanced activities of all the three lysosomal enzymes up to first 30 days of the extract treatment. In last 30 days of treatment, except for the activities of nonspecific esterase, the activities of the remaining two lysosomal enzymes were increased. Since there was insignificant effect on Leydig cells, these enzymatic changes could not be attributed to androgenic imbalance. Such a behaviour of enzymes was indicative of degenerative changes. Part of the
enzymatic alterations also appeared to be due to alterations in the mucosal cells as seen from histochemical observations.

3) Alkaline phosphatase:

Biochemically as well as histochemically the alkaline phosphatase in treated rats did not show any significant changes.

4) Mucopolysaccharides:

The mucosa, muscular coat and secretion of seminal vesicles of treated rats showed increase in all mucosubstances over the control. Comparatively the secretion of seminal vesicles showed more increase in the mucosubstances. The Butea leaf extract treated seminal vesicles of rats elaborated more amounts of the mucosubstances and secreted them into lumen.

5) Lipids:

Though not significant, an overall increase was observed in total lipids, neutral lipids and phospholipids including all their components. Earlier literature on the effects of treatment with antiandrogenic substances, shows a depletion in the lipids of seminal vesicles. But the observations of Butea leaf extract treatment showed increase in most of the lipids, thus indicating that the action of Butea leaf extract is not antiandrogenic in nature.

6) Proteins:

The total proteins did not exhibit any remarkable alterations after administration of Butea leaf extract. The observations indicate that the extract exerted very little effects on the structure of seminal vesicles of treated rat.
IV. Prostate gland:

1) Alterations in wet weight and histology:

The administration of extract caused an increase in the wet weight of prostate gland. Such increase seemed to be due to concentration and accumulation of the secretion in the acini due to extract treatment. Very insignificant reduction in the height of acinar epithelial cells of prostate gland was noticed. The interacinar tissue appeared normal except for little thickening in basal lamina. The secretion was concentrated and was seen attached to inner lining of acini.

2) Lysosomal enzymes:

At the termination of Butea leaf extract treatment β-glucuronidase showed three fold increase, acid phosphatase two fold increase, and nonspecific esterase showed insignificant decrease. Histochemically between 45th to 90th days of the extract treatment the activities of β-glucuronidase and acid phosphatase in the acinar cells and interacinar tissue showed enhancement, while nonspecific esterase in these structures exhibited decreased activity.

3) Alkaline phosphatase:

The alkaline phosphatase alterations involved an initial decrease, then increase in middle phase of extract treatment followed by decrease in the activity in final phase of treatment. Histochemically the staining intensity was as good as in control. Thus the alkaline phosphatase and acid phosphatase alterations were antagonistic in nature, which was indicative of cytotoxic action of Butea leaf extract. But actually in the histological and histochemical observations there was no evidence of cytotoxic action at light microscopic level. Probably it might be occurring at ultrastructural level.
4) Mucopolysaccharides:

In the phase of 45th to 90th days of the extract treatment, the histochemical reactivities indicated reduced amount of glycogen and sialic acid in acinar cells, interacinar tissue and secretion, but neutral mucopolysaccharides were increased in the secretion of acini. The changes in mucosubstances could not be attributed to androgen imbalance. They might be due to functional alterations in the extract treated rat prostate.

5) Lipids:

_Butea monosperma_ leaf extract treatment depleted the values of total lipids, neutral lipids and phospholipids including all their components. The observed depletion in the lipids in prostate might be either due to their degradation or inhibition of their biosynthetic pathways in the treated rat prostate.

6) Proteins:

The _Butea_ leaf extract caused no significant changes in the total proteins of prostate gland. There was an initial increase in protein values upto 30 days and then they depleted and were practically near the control values.

V. Cowper's gland:

1) Alterations in wet weight and histology:

The _Butea_ leaf extract induced alterations in wet weight of Cowper's glands were not significant and there was no consistency in the changes. No atrophy of the gland was in evidence, neither the epithelium showed any significant involution. These observations indicated that the _Butea_ leaf extract did not exert any antiandrogenic action in the treated rats.
2) Lysosomal enzymes:

The Cowper's gland showed increase in β-glucuronidase and nonspecific esterase activity. The acid phosphatase exhibited very insignificant alterations. These changes in these hydrolysing enzymes suggested that there were less necrotic or degenerative changes in the Cowper's glands of the treated rats.

3) Alkaline phosphatase:

The alkaline phosphatase of Cowper's gland increased during the period of the extract treatment.

4) Mucopolysaccharides:

The treatment of Butea leaf extract resulted in the reduction of glycogen, PAS unreactive sulfomucins and sialomucins and diastase resistant PAS reactive mucopolysaccharides in the epithelial cells, increase in PAS unreactive sulfomucins and sialomucins and PAS reactive diastase-resistant neutral mucins in stroma, and decrease in the diastase resistant PAS reactive mucosubstances and PAS unreactive sulfomucins and sialomucins in the secretion of Cowper's gland.

5) Proteins:

Butea monosperma leaf extract administration to albino rats resulted in an increase in the total protein values of Cowper's gland.

Concluding Remarks:

Butea monosperma leaf extract induced alterations in the male reproductive tract of the albino rats studied in the present investigations show that the testes are the main target organs of the active principle in the extract, whereas the epididymis forms a minor target organ of the extract. The other sex accessory organs such as seminal
vesicles, prostate and Cowper's gland do not exhibit any marked structural changes, but at enzymatic and metabolic level they do exhibit some changes. Hence these accessory organs may not be forming primary target organs of the *Butea* leaf extract. Whatever enzymatic and metabolite changes that are seen in these organs may be indirect in nature. Not only a proper androgenic level is needed for the perfect functioning of these organs, but entry of viable normal spermatozoa through the sperm duct, into which the ducts of these organs open, is also needed for the proper functioning of these organs. Hence the changes seen in these organs may also be due to the depleted number of normal viable spermatozoa in the sperm duct of the *Butea monosperma* leaf extract treated rats.

The present investigations open several avenues for further research on the *Butea monosperma* leaf extract induced aspermatogenesis and concomitant changes in the accessory glandular and nonglandular organs of male genital tract. Some ideas for such further work are given here:

1) In the present investigations it is concluded that the effects seen are not due to any androgenic imbalance. But this conclusion is only an indirect inference of the observations. To confirm or modify the above conclusion, bioassay studies of androgen in the treated rats are highly desired.

2) The histological observations of testicular alterations show no significant alterations in the Leydig cells. From this it has been inferred that the Leydig cell functioning is not affected in the treated rats. Studies involving steroid dehydrogenase activity in the Leydig cells might have given some reliable data on the function of the Leydig cells in the treated rats. But it was not
done in the present investigation for want of substrates of these enzymes.

iii) Due to antagonistic behaviour of acid and alkaline phosphatases in some sex accessories, the possibility of presence of cytotoxic agents is suspected in Butea leaf extract. Hence it is highly desirable to find out whether such cytotoxic action occurs in physiologically important organs such as kidney, liver, brain etc. Such teratological studies should form a condition for all investigations involving action of drugs, chemicals and plant extracts on male or female reproductive tracts. Some work in this direction is in progress in this laboratory.

iv) In order to have more insight into the present observations, the biochemical studies of fructose, citric acid and sialic acid in the testes and sex accessories are also desired.

v) To get a general picture of possible changes in the physiology of body as a whole, it is necessary to study the serum enzymes and proteins in the treated rats. This will also give some information on the side effects of the Butea leaf extract treatment.

vi) In the present investigation many well known and recent available techniques have been used. But to get a thorough insight into alterations, application of better techniques is desired. Some such techniques are: (i) Observations of testicular changes especially in various cell types by electron microscopy. (ii) Separation of testicular mitochondria, microsomes, lysosomes, nuclei etc. by ultra-centrifugation and subsequent biochemical studies. (iii) Abnormalities in DNA of different cell types by fractionation and gel electrophoresis. (iv) Appearance or disappearance of special proteins in reproductive organs by gel electrophoresis.
vii) It will be very interesting to find out whether the extract induced aspermatogenic changes can be reversed by administration of hormones such as FSH. Practically nothing is known about changes in pituitary caused by the Butea leaf extract. Hence study of pituitary cell types in the treated rats may also reveal some interesting information.

While concluding the present Doctoral dissertation, the author would like to state that the present work is by no means complete. He is fully aware of his shortcomings. The effect of various doses of the extract should have formed a part of the present thesis, but this has not been done since it would have made this thesis still more elaborate. The author feels gratified that he has made a detailed histological, biochemical and histochemical study of the male reproductive tract during induced aspermatogenesis in Butea leaf extract administered rats, made some original contributions which have not been reported and viewed the observations comparatively to arrive at some conclusions regarding the mode of action of the antispermatogenic agent. There is unlimited scope for further work on induced aspermatogenesis caused by Butea leaf extract. Some work on the problems outlined above is in progress in this laboratory, which, it is hoped, might throw more light on the possible use of Butea monosperma leaf extract as a male contraceptive agent having minimal side effects.