CHAPTER V

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The present study was carried out with special reference to the following:

1. To test the efficacy of papaya seed extracts viz., benzene, butanol or methanol alone and in combination (benzene:butanol:methanol in the ratio 1:1:1) as contraceptive agents on male mice (Mus musculus) and male rats (Rattus norvegicus).

2. To study the effects of papaya seed extracts on the structure (histology and ultrastructure) and function of reproductive organs of male mice and rats.

3. To investigate, if these papaya seed extracts had any side effects on the structure and metabolism of some vital organs. The estrogenicity and LD_{50} was also checked.

STUDIES ON FERTILITY REGULATION AND ALTERED FERTILITY STATUS IN SOME MALE MAMMALS

Healthy, adult male albino mice (Mus musculus) and albino rats (Rattus norvegicus) were administered different papaya seed extracts [benzene, butanol, methanol, benzene:butanol:methanol (1:1:1)] at a dose of 20 mg/kg body weight for 45 days to mice and 60 days to rats. The control and treated animals were maintained on a standard chow and water ad libitum. The treated animals i.e. mice were sacrificed
The results revealed that:

1. No significant changes were observed in the body and organ weights of all the experimental groups of animals. Serum electrolyte balance remained unaffected by the extract treatments as serum Na\(^+\), K\(^+\) and Ca\(^{2+}\) levels remained unaltered.

2. The extract treatments did not reveal any estrogenic effects since, estrogen dependent changes in ovariectomised immature female mice were not observed in treated mice when compared to the standard estradiol treated groups of animals.

3. The study also elucidated that all the papaya seed extracts did not affect testis structure, spermatogenesis and steroidogenesis as is evident from the unaltered sperm counts and levels of 3β HSD, 17β HSD, cholesterol, serum FSH, LH and testosterone.

4. The extracts altered the histology of epididymis. The epididymal tubules showed confluences due to apical degeneration. The metabolic status of the epididymis was also affected by different extract treatments. The activities of SDH, ATPase, levels of protein and sialic acid in the caput and cauda epididymides were significantly decreased.

5. The transmission electron microscopy of the cauda epididymal sperm of all the
extract treated groups revealed head abnormality and presence of cytoplasmic droplet. The integrity of the plasma and the acrosomal membranes of spermatozoa were impaired. That the acrosomal integrity was affected by the extract treatments was also evident by the silver nitrate staining.

6. The different extract treatments significantly altered sperm acrosomal enzymes viz. acrosin and hyaluronidase which are a prerequisite prior to fertilization and therefore a decline in these enzymes could result in the poor penetrating and fertilizing ability of the spermatozoa leading to reduced fertility. Any damage to the acrosome may result in the loss of enzymes rendering the sperm non-viable which is further supported by the fact that there was significant decline in the percentage of live sperms following different extract treatments.

7. The cauda epididymal sperm motility of the treated animals was significantly reduced. Qualitative motility was greatly affected in all the extract treated groups as evidenced by impaired forward progression.

8. Correlating with reduced motility and forward progressive motility, a significant decrease was noted in sperm mitochondrial activity index in all the treated groups of mice.

9. All the extract treatments brought about a significant reduction in the fertility rate of normally cycling females with treated males. The reduction in the fertility could be correlated with the significant decrease in percent cauda epididymal sperm motility, loss of viability, decrease in the activities of
acrosomal enzymes, acrosomal damage and abnormal sperm morphology.

10. The vas deferens of all the extract treated groups of mice did not reveal much alterations in its histology except that slight nuclear pyknosis was observed in the methanol extract treated group. The glycogen concentrations were increased in all the experimental groups of animals whereas the activity of phosphorylase declined significantly suggesting that carbohydrate metabolism and secretions of the vas deferens were altered after treatment.

11. No significant changes were observed in the levels of fructose in seminal vesicles in all the extract treated groups of animals.

12. The levels of RNA and DNA in the testis, caput and cauda epididymides of the extract treated groups of mice were not affected elucidating that nucleic acid metabolism remained unaltered which was further supported by the fact that the nuclear integrity of the epididymal sperms of mice was unaltered throughout the different extract treatments as revealed by acridine orange staining.

13. The study also elucidated that all the extract treatments did not manifest any toxic side effects. Unaltered histology of liver and kidney and serum biochemical parameters viz., SGPT, SGOT, protein, cholesterol, creatinine, acid phosphatase and alkaline phosphatase indicate lack of toxic effects of seed extracts on vital body organs and general health of the animals. The LD₅₀ studies also revealed that the extracts were non toxic.

14. In order to ascertain the reversibility of the induced effects in mice, the
treatment was withdrawn for a further period of 45 days after 45 days of treatment. In rats the treatment was withdrawn for 60 days after 60 days of treatment and the data revealed significant recovery in all the induced effects.

15. The fertility rate was restored back to normal suggesting that effects of the extract treatments were transient and reversible after the withdrawal of the treatment.

Therefore, functional sterility could be induced in male mice and rats by all the extract treatments.

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

(a) All the papaya seed extracts on oral administration were effective in bringing about a contraceptive effect.

(b) All the extracts lacked estrogenic effect.

(c) The extracts tested were non-toxic as revealed by the LD$_{50}$ value of 18 and 20 g/kg body weight which is much higher than the LD$_{50}$ dose of 5 gm/kg body weight recommended by the WHO as of non-toxic nature for a plant product.

(d) Impaired sperm motility, viability, sperm abnormality, acrosomal damage, activities of sperm acrosomal acrosin and hyaluronidase along with altered epididymal biochemical parameters were evident after the different extract treatments suggesting that the extracts interfere with the physiological functioning of the epididymis. The above mentioned changes resulted in a
100% negative fertility rate.

(e) The extracts probably disrupt the -SH groups of protein and form -SS linkages causing alterations in the sperm membrane particularly those of acrosome.

(f) All the induced effects were transient and reversible after the withdrawal of the treatments.

Hence, it could be concluded that different papaya seed extracts were effective at very low doses, were non-estrogenic, non-toxic, had reversible antifertility effects and thus proved to be effective male contraceptive agents in rodents.

**FUTURE LINE OF WORK**

1. Receptor studies to detect if their concentration or configuration is altered.

2. Study of 5α-reductase activity in epididymis since testosterone levels were not affected but target organ response to the androgen was decreased.

3. Isolation, characterization and identification of the active component(s) in the papaya seed extract and testing of their biological activity. Some studies in this direction are being carried out.

4. Since the contraceptive efficacy of the papaya seed has been established and tested in different rodents, Phase I trials should be initiated to test their effect in non-human primates. This is very important since these extracts are effective as antifertility agents also in female rodents. Hence a unisex contraceptive could be formulated for the first time.