CHAPTER IV

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The efficacy of papaya seed extracts alone or in combination viz., benzene, butanol, methanol, mixture of benzene:butanol:methanol (1:1:1) as contraceptive agent was tested on male albino mice (Mus musculus) and male albino rats (Rattus norvegicus). The extracts were administered orally at a dose of 20 mg/kg body weight for 45 days to mice and for 60 days to rats.

Earlier work from our laboratory (Chinoy and Sam George, 1983; Chinoy and Geetha Ranga, 1984; Chinoy et al., 1984/85; 1994; 1995a,b; Joshi and Chinoy, 1996; Chinoy and Padman, 1996; Chinoy et al., 1997a,c,d) has revealed that different doses used viz., 0.1, 1, 5, 10, 15 and 20 mg/kg body weight/day/animal of aqueous, alcoholic and benzene extracts of papaya seeds were potent in reducing fertility in both sexes of rats and mice. The dose of 20 mg/kg body weight/day/animal was most effective in causing fertility impairment and therefore this dose was selected for the present investigation.

Various routes for administration of the extracts i.e. intramuscular, subcutaneous, gastric intubation and oral have been tried and all these routes of administration were found to be effective in both males and females for inducing functional sterility. However, in the present study, the oral route was selected for the treatment which is an easy and feasible way to administer the extract.
In our laboratory, seeds of four different varieties of papaya have already been tested, viz. Honey Dew, Ceylon, Ranchi Dwarf and Washington. Amongst these four varieties, Honey Dew was found to be the most effective as an antifertility agent in male rats (Chinoy et al., 1996). Therefore in the present work the seeds of Carica papaya variety Honey Dew have been utilised.

The various parameters studied at the end of the treatments were estrogenicity checking of methanol and butanol papaya seed extracts, LD₅₀ studies, whole body and organ weights, histology and ultrastructure of reproductive organs.

The parameters considered for assessing the antifertility effect of the benzene, butanol, methanol, benzene:butanol:methanol extracts were sperm motility and count, viability and fertility rate. Some sperm functional tests like sperm mitochondrial activity index, acidic alcoholic silver nitrate staining and acridine orange staining was also carried out to assess acrosomal and nuclear integrity. Certain biochemical parameters viz., cholesterol, activities of 38 and 178 hydroxysteroid dehydrogenases, serum FSH, LH and testosterone levels were determined to study the effect of papaya seed extracts on testicular steroidogenesis. The sperm acrosomal enzymes, acrosin and hyaluronidase were assayed as they are important enzymes for the acrosome reaction and fertilisation. In addition, the concentration of glycogen, activity of phosphorylase were studied in vas deferens. The levels of DNA, RNA and protein were determined in testis, caput and cauda epididymides to study the effects of the treatment on nucleic acid metabolism in these organs. The levels of serum Na⁺, K⁺ and Ca⁺⁺ ions were also
investigated using flame photometry. Some selected parameters were investigated to study the toxicological effects, if any.

In a different set of experiments, mice were treated with the extracts for 45 days and thereafter the treatment was withdrawn for another 45 days to study the reversibility, if any, of the induced effects. The rats were treated with extracts for 60 days and thereafter the treatment was withdrawn and the reversibility was studied after a period of further 60 days.

ESTROGENICITY CHECKING OF PAPAYA SEED EXTRACTS

As required by the WHO standards, the extracts (methanol and butanol) were first tested for their estrogenicity, if any, according to the WHO protocol MB-70/71. Ovariectomised, extract treated immature female mice did not reveal any estrogen dependent changes such as increase in body weights or absolute uterine and adrenal weights, or the presence of vaginal opening. The study revealed that the methanol and butanol extracts of papaya seeds were non-estrogenic when tested in immature albino female mice. Moreover, the earlier work from our laboratory and elsewhere has elucidated that the aqueous, alcoholic, benzene and hexane papaya seed extracts are devoid of any estrogenic activity (Chinoy et al., 1994; Padman, 1995; Chinoy and Padman, 1996; Keshri et al., 1993). Lohiya and Goyal (1992) reported that crude chloroform extract was mildly estrogenic in nature. However, this study was carried out by an estrogenic bioassay and not by standard WHO Protocol. An estrogenic effect
is an undesirable effect in the formulation of a male contraceptive agent, since estrogens are known to manifest anti-androgenic and anti-gonadotrophic effects in the males, besides causing certain unwanted side effects. Estrogens are also known to inhibit testicular and accessory sex gland functions (Bartke et al., 1977; Johnson and Gomes, 1977; Chinoy and Rao, 1982; Chinoy et al., 1982; Rao and Chinoy, 1984; Chinoy et al., 1984). Besides, the antifertility effects of most of the plant products is ascribed to their estrogenicity. Therefore, the fact that both methanol and butanol extracts do not possess estrogenic effect, is of advantage.

LD$_{50}$ studies revealed that both methanol and butanol papaya seed extracts were non-toxic. The LD$_{50}$ dose was 18 and 20 g/kg body weight which is much higher than the LD$_{50}$ dose of 5 g/kg body weight recommended by WHO as of non-toxic nature for a plant product. Earlier studies have reported similar results in aqueous, alcohol and benzene papaya seed extracts (Chinoy et al., 1994; Padman, 1995; Joshi, 1995) in corroboration with the present data. The body weights of all the treated animals (mice and rats) were not affected following different extract treatments.

The weight of cauda epididymis in the methanol treated groups of mice and in benzene treated group of rats was decreased. However, the weights of testis, caput epididymis, vas deferens, liver and kidney were not affected. This indicated that the extracts do not promote weight gain causing obesity and water retention. Similar result was obtained by Das (1980) in male rats by treatment with papaya seed extract. Moreover, earlier work carried out in our laboratory has also elucidated that during
administration of different extracts of papaya seeds in male and female rodents, the
body and organ weights were not affected (Chinoy and Sam George, 1983; Chinoy
and Geetha Ranga, 1984; Chinoy et al., 1984/85; 1994; 1997a,d, Chinoy and Padman,
1996). Lohiya et al. (1994) observed a significant decrease in the testicular weight
after administration of aqueous extract to male rats. However, the treatment had no
significant effect on the body weight and weights of epididymis, vas deferens and
adrenal in corroboration with the present data.

Maintenance of electrolyte balance is important as changes in the level of
sodium and potassium may cause loss of water from the cells and tissues leading to
reduction of body weights. Alterations in serum calcium levels would affect the
various reactions catalysed by the enzymes activated by calcium and numerous
calcium dependent functions of the body. Sondarva (1989) has shown unaltered serum
Na⁺ and K⁺ levels following aqueous extract of papaya seed treatment. Chinoy et al.
(unpublished observations) have also found unaltered Na⁺ and K⁺ levels in kidney after
alcoholic extract treatment to rats which is in agreement with the present data. Thus
the data suggests that neither water retention occurred in the body, nor electrolyte
balance was disturbed. The maintenance of ionic balance is important since studies on
an earlier contraceptive agent (Gossypol) was found to cause hypokalemia in human
males (Prasad and Diczfalusy, 1983). It is known that serum protein levels play an
important role in maintaining osmotic balance and any alteration would cause oedema.
However, in the present study, no such changes were obtained in confirmation with
earlier studies carried out on male mice and rats (Chinoy et al., 1984/85; 1994).

The present investigation revealed in conformity with the findings of the earlier work carried out in our laboratory with aqueous extract, that all the extract treatments did not affect the histoarchitecture of the testis of rats (Chinoy and Sam George, unpublished observations) and mice (Chinoy and D’Souza, unpublished observations). Different stages of spermatogenesis as well as luminal sperms were observed. Hence spermatogenesis occurred unabated. The morphology of the Leydig cell was not affected by the different extract treatments indicating that the paracrine functions of the testis were not affected.

The activities of 3β hydroxysteroid dehydrogenase (HSD) (which converts dehydroepiandrosterone to androstenedione) and 17β hydroxysteroid dehydrogenase (which converts testosterone to androstenedione) which are key enzymes in steroidogenic pathway in testis, were not altered following the different extract treatments. The cholesterol levels in the testis also remained unaffected. The normal serum testosterone levels in all the treated groups of mice corroborate with earlier data (Chinoy and Sam George, 1983; Chinoy et al., 1984/85; Chinoy and Padman, 1996) and suggest that the testicular steroidogenesis was unaltered after treatments. This is further confirmed by the fact that in the present study the sperm count was not affected following different extract treatments in both rats and mice in corroboration with earlier data (Chinoy and Sam George, 1983; Chinoy et al., 1984/85; 1994; Chinoy and Padman, 1996) which suggested that the spermatogenesis was not altered.
On the contrary, Lohiya et al. (1994) have reported a significant decline in testicular sperm counts in rats following aqueous extract treatments. They attributed this decrease in sperm count to the antispermatogenic nature of the extract.

The serum FSH and LH levels were also within the normal range which is again in accordance to earlier data (Chinoy and Sam George, 1983; Chinoy et al., 1984/85; Chinoy and Padman, unpublished observations). Therefore it is evident that the hypothalamo-pituitary - gonadal axis is not altered elucidating that the extracts had no antigonadotrophic effects which supports our data that the extracts are non-estrogenic.

Based on these observations, it is clear that all the extracts have no effect on spermatogenesis, steroidogenesis nor the hypothalamo-pituitary gonadal axis.

Epididymis is an important organ in the male reproductive system and is the site for sperm maturation (Bedford, 1975; Prasad and Rajalakshmi, 1976; 1977; Chinoy, 1984; Hamilton, 1975; Robaire and Hermo, 1988; Cooper, 1992; 1993; Robaire and Viger, 1993). Epididymis contributes to the physiological maturation of the spermatozoa through secretion of several proteins and glycoproteins/and modifying some of the surface proteins with which the spermatozoa reach ductus epididymis. The microenvironment of the epididymal ductal lumen changes constantly from the initial segment to the distal cauda, and it contributes to the physiological maturation of the spermatozoa during their passage along the epididymal duct (Robaire and Hermo, 1988). Therefore any direct or indirect effect of a drug/chemical on the epididymis
might render the spermatozoa immotile and/or non-fertilizable.

In the present investigation, the light microscopy and ultrastructural studies of the caput and cauda epididymides of mice subjected to different extracts of papaya seeds were carried out. As the histology of testis was unaffected in treated mice, the EM was not done.

The caput and cauda epididymides of the mice treated with different papaya seed extracts showed tubular confluences due to apical degeneration and loss of basement membrane. Hence the diameter of the tubules were enlarged. The nucleus showed pyknosis at the apex of the tubules where degeneration occurred which corroborate with similar changes observed earlier (Chinoy and D’Souza, unpublished observations, Chinoy and Geetha Ranga, unpublished observations).

The scanning electron microscopic observations of cauda epididymal spermatozoa of animals treated with aqueous extract had revealed abnormal morphology. The sperms had head abnormality, were deflagellated, coiling of tail and end piece and presence of cytoplasmic droplet were also observed (Chinoy and Sam George, 1983; Chinoy et al., 1995b).

In the present study, transmission electron microscopy of the cauda epididymis treated mice revealed no major changes in the epithelium. However, the sperms of all the extract treated groups revealed head abnormality. The integrity of the plasma and acrosomal membranes of the spermatozoa were impaired. The sperms of all the extract treated groups showed presence of cytoplasmic droplet.
Large number of sperms retained the cytoplasmic droplet. The cytoplasmic droplet is a small mass of cytoplasm that is retained by the spermatozoa after its release into lumen of the seminiferous tubules. In the seminiferous tubules and efferent ducts, the droplet remains in the neck region. In the caput epididymis, it is seen near the junction between the mid and principal piece of the flagellum. The droplet shows a lateral displacement when sperms are in the corpus and it is pinched off on or before arriving at the cauda epididymis. Once detached the droplet quickly breaks up, thereby releasing its contents which are endocytosed by the clear cells of the cauda epididymis to be digested by their secondary lysosomes (Hermo et al., 1988). Thus most of the sperms in the cauda epididymis must be devoid of the cytoplasmic droplet. Ejaculates containing a high proportion of spermatozoa with attached droplet could be correlated with altered epididymal milieu and hence function as well as reduced fertility (Cummins and Glover, 1970; Cummins, 1973; Bedford, 1976). Spermatozoa carrying the cytoplasmic droplet would have inhibited motility and hence may not be fully viable and fertilizable (Hermo et al., 1988).

The metabolic status of the epididymis was also affected by different extract treatments. The activity of ATPase, an androgen dependent enzyme was significantly decreased in caput and cauda epididymis of all the extract treated groups of mice and rats. A similar inhibition of ATPase activity in epididymis and epididymal sperms was also obtained by Chinoy et al. (1984/85; 1994). The reduction in ATPase activity might be a contributory factor for the decrease in sperm motility, since the enzyme
plays an important role in the energy metabolism of spermatozoa.

Succinate dehydrogenase is an oxidative enzyme which is involved in Krebs cycle. In the present study, a decrease in SDH activity in caput and cauda epididymides of mice and rats after different extract treatments is indicative of alterations in the oxidative metabolism of the epididymis. Earlier studies carried out by Chinoy et al. (1984/85, 1994) also reported a decrease in the activity of SDH.

Sialic acids are important constituents of mucopolysaccharides and sialomucoproteins. Sialic acids are secreted by the epididymal epithelium and are coated on the spermatozoa as they pass through the epididymis. They are concerned with changing the membrane surface of maturing spermatozoa, in development of their fertilizing capacity and involved in stabilization of the acrosome and its membrane during sperm maturation (Hamilton, 1977; Rajalakshmi et al., 1976). In the present study, a significant decrease occurred in the levels of sialic acids in epididymis in all the extract treated groups of rats and mice. A similar significant decline in sialic acid concentration of epididymal spermatozoa and luminal fluid of the aqueous treated rats has been reported earlier (Chinoy et al., 1997d). These results are further corroborated by electron microscopic studies of the epididymal spermatozoa. The spermatozoa of all the extract treated mice showed various degrees of loss of the integrity of the membranes of the acrosome. That the acrosomal integrity was affected by the extract treatment was also evident by the silver nitrate staining.

The extract probably disrupts the -S-S linkage of proteins causing alterations
in sperm membrane particularly those of acrosome. The role of acrosome in fertilisation is well established as it contains a number of enzymes such as acrosin, hyaluronidase, B-glucoronidase, acid and alkaline phosphatases which play a significant role in egg penetration. Acrosin and hyaluronidase are two principal enzymes required for acrosomal reaction before fertilisation. Acrosin a neutral proteinase is instrumental in the penetration of zona pellucida and cervical mucus by the spermatozoa (McRorie and Williams, 1974; Schumacher and Zaneveld, 1974). It occurs partly in the form of an inactive zymogen precursor, proacrosin (Polakoski et al., 1977). Assay of acrosin therefore, involves assay of zymogen precursors and activation of acrosin after removal of inhibitors, to quantitate total acrosin (Polakoski et al., 1977; Bhattacharya and Zaneveld, 1978). In the present study, proacrosin levels increased following different extract treatments while a decrease occurred in free acrosin and acrosin-acrosin inhibitor complexes. This suggests a block in autoactivation of proacrosin. Schill et al. (1988) have reported that activation of proacrosin levels might be due to alterations in glycosaminoglycans (GAG) concentration which are known to stimulate the conversion of proacrosin into its active forms (Parrish et al., 1980). The low sperm membrane bound acrosomal enzymes have been reported to be associated with morphological abnormality or acrosomal damage (Schill, 1974). Hyaluronidase disperses the cumulus oophorus and the spermatozoa uses their enzyme in penetration of the outer most layer of the ovum (Zaneveld et al., 1973). Hyaluronidase is also associated with the acrosome of the spermatozoa.
A significant reduction in the hyaluronidase activity obtained after the different extract treatments could be related to low penetrating activity of sperms and subsequently reduced fertility. Amelar and Dubin (1979) reported decrease in sperm acrosomal enzymes as one of the reasons for the lower fertilizing capacity of semen. Hence any damage to the acrosome may result in loss of enzyme rendering the sperms non-viable. A significant decrease in the acrosomal enzymes, hyaluronidase and acrosin obtained in the present study is in confirmation with the above observations and therefore the extracts rendered the sperms non-viable. This is further supported by the fact that there was a significant decline in the percentage of live sperms following the different extract treatments which in turn reduced the fertility in accordance with earlier data of Chinoy and Padman (unpublished observation) with benzene and alcoholic extracts in male rats.

In the present study, cauda epididymal sperm motility declined significantly in all the extract treated groups of mice and rats. This decline in sperm motility is in conformity with earlier reports from our laboratory (Chinoy and Sam George, 1983; Chinoy et al., 1984/85; 1994; 1997d; Chinoy and Padman, 1996; unpublished observations). Qualitative motility was greatly affected in all the extract treated groups in the present study as evidenced by impaired forward progression, where the spermatozoa showed wriggling movement. Assessment of sperm motion characteristics by computer assisted semen analysis (CASA, Cellsoft 2000, USA) revealed a significant decline in the forward progressive motility of sperms of rats treated with
alcoholic and benzene extracts of papaya seeds (Chinoy and Padman, 1996). Lohiya et al. (1992; 1994; Lohiya and Goyal, 1992) also reported a significant decline in motility of epididymal spermatozoa of rats following papaya seed extract treatments. Lack of forward progressive motility is one of the factors resulting in reduced fertility because only actively forward progressive motile spermatozoa are capable of penetrating and ascending the female reproductive tract. The type of movement of the sperm thus influences the fertilizing capacity. Sperms swimming in tight circles cannot readily pass through the uterotubal junction, and only straight swimmers succeed in fertilizing the egg (Blandau and Rumery, 1964). Correlating with reduced motility and forward progressive motility, a significant decrease was noted in sperm mitochondrial activity index (SMAI) in all extract treated groups of mice. All the extract treatments brought about a significant reduction in the fertility rate of normally cycling female animals mated with treated males, fertility being assessed by counting the number of implantation sites about 16 days after mating. The reduction in 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. Hafez (1976; 1977) observed that abnormal sperm morphology is one of the factors for causing male sterility.

Chinoy et al. (1994) reported a fall in protein levels of epididymal sperms after aqueous extract treatment to mice. The PAGE study of cauda epididymal proteins indicated that the aqueous extract treatment brought about alterations in the different
protein fractions. Brooks (1984) reported that a restricted energy supply modifies protein secretion by creating a disturbed intracellular Na/K balance as well as inhibition of Na/K dependent ATPase activity. Thus reduction in ATPase activity, especially in cauda epididymis might be responsible for the change in protein fractions of the epididymis in treated mice (Chinoy et al., 1994). In the present study a significant decrease in the levels of proteins of epididymis was noted in all the extract treated groups of rats and mice which could be one of the causative factors for the reduction of sperm motility (Hoskins et al., 1978). Lea et al. (1978) suggested that glycoproteins secreted by the epididymis are coated on the sperm surface, which are capable of stimulating motility in bull spermatozoa (Brandt et al., 1978).

Similarly, the protein levels declined in testis and vas deferens of all the extract treated groups of mice and rats. However, the levels of protein in liver and kidney remained unaffected in all the experimental groups of mice and rats. Earlier studies (Chinoy and Sam George, 1983; Chinoy and Geetha Ranga, 1984) carried out in our laboratory revealed that the treatment with aqueous Carica papaya seed extract to rats produced changes in the vas deferens physiology, contractile pattern and alteration in the muscle layer thickness. The effect of the treatment on the various androgen sensitive parameters of the vas deferens was also similar to that manifested for the caput and cauda epididymides. The authors suggested that the reduced/ altered contractile response of the vas deferens in treated rats might be a contributing factor in reducing fertility.
In the present study the vas deferens of all the extract treated groups of mice did not reveal much alterations in its histology. However, vas deferens of the animals treated with methanol papaya seed extract showed slight nuclear pyknosis. The histocytometric studies revealed no significant changes in the epithelial cell height. However, the muscle layer thickness was decreased.

The glycogen concentrations were increased in vas deferens of all the extract treated groups of mice and rats. The increase in glycogen concentration could be correlated with the decrease of phosphorylase activity in the vas deferens and indicates that glycogen metabolism might be affected.

The biochemical markers known for the evaluation of vesicular function are fructose, prostaglandins, and several basic proteins, but so far only fructose has been used for evaluation of the secretory capacity (Mann, 1964; Eliasson, 1965; Hammarstein and Brotherton, 1978 and Abyholm et al., 1981). In the present study the levels of fructose in seminal vesicles did not show significant changes after different extract treatments in both rats and mice which supports the observation that the extracts have no anti-androgenic effect.

The RNA and DNA levels in the testis, caput and cauda epididymides of the extract treated groups of mice were not affected suggesting that nucleic acid metabolism remained unaltered. Moreover, the papaya seed benzene extract did not affect the frequency of sister chromatid exchange or cause chromosome aberrations in human leucocytes in vitro (Chinoy et al., 1997b). Furthermore, the sperm nuclear
integrity of the epididymal sperms of mice was also unaltered throughout the different extract treatments as evident from the unaltered levels of percent green-fluorescing sperms as compared to control animals after acridine orange staining.

The studies showed that the histology of liver and kidney as well as their metabolism were not affected in all treated groups of animals.

It is known that in a normal liver cell, glutamate oxaloacetate transaminase (GOT) is localised in the mitochondria and cytoplasm whereas, glutamate pyruvate transaminase (GPT) is localized in cytoplasm (Merck, 1974b). Increase in the level of these enzymes in the serum/plasma indicates hepatocellular death or damage caused by hepatotoxins which enhance the hepatic cell membrane permeability leading to the release of these enzymes in the blood stream (Hess, 1962). However, such changes were not found in the present study, since the levels of SGPT and SGOT in the serum of the treated mice and rats were within the normal range in support of the earlier work (Chinoy et al., 1984/85; 1994; 1997b; Lohiya et al., 1992; Lohiya and Goyal, 1992). The histology of liver also revealed no alterations in papaya seed extract treated mice. These observations were further supported by the normal levels of glycogen, activity of phosphorylase, activities of acid and alkaline phosphatase and protein in the liver which suggests that the extract treatments had no adverse effects on the structure and functions of liver. Similarly the histology of kidney, its protein, activities of acid and alkaline phosphatase and creatinine levels, along with serum creatinine levels were not affected. Hence the structure and functions of the kidney was not altered in the
treated animals.

Total serum cholesterol levels are known to be raised in cases of hypercholesterolemia, hyperlipidemia, hyperthyroidism and obstructive jaundice. However, in the present study the serum cholesterol remained unchanged in all the treated groups of mice in agreement with earlier work (Chinoy et al., 1984/85; 1994; 1997b) which indicates that its metabolism was not affected. This observation is further supported by the fact that testicular cholesterol levels, and serum testosterone levels were also not affected.

The LD$_{50}$ dose was much higher than the dose of 5g/kg body weight recommended by the WHO as of non-toxic nature for a plant product. This revealed that the papaya seed extracts used were non-toxic. The non-toxic nature of other papaya seed extracts viz., aqueous and alcoholic has already been established (Chinoy et al., 1994; Chinoy and Padman, unpublished observations) and other workers (Lohiya and Goyal, 1992; Lohiya et al., 1994) have also reported the non-toxic nature of aqueous and chloroform papaya seed extracts.

As mentioned earlier, though the testosterone levels were not affected after the various extract treatments, the metabolism of androgen-dependent target organs was altered especially the cauda epididymis. This androgen deprived effect may be due to the reduced conversion of endogenous testosterone to its active metabolite, 5α-DHT by the probable reduction in 5α-reductase activity or else a change in the configuration or concentration of receptors in target organs. It follows that a low target organ
response to androgens will occur. These aspects need to be studied in detail.

The mechanism of action of papaya seed extracts at a dose of 20 mg/kg body to mice seems to be through a direct action on the sperm by altering its membrane structure. The head and tail region of sperm are rich in sulphhydryl groups. The extract probably disrupts -S-H groups of protein and forms -S-S linkages causing alterations in the sperm membrane particularly those of the acrosome as mentioned earlier. The alteration in the contractile pattern of the vas deferens might also be a contributing factor for reduction in fertility.

In order to investigate reversibility of the induced effects by the treatment, a group of animals, i.e. mice were treated with extracts or 45 days and thereafter the treatment was withdrawn and the reversibility was studied after a period of 45 days. On the other hand, the rats were treated with the extracts for 60 days and thereafter the treatment was withdrawn and the reversibility studied for a further period of 60 days. The findings revealed that all the induced effects, i.e. reduction in sperm motility, the biochemical parameters assessed as well as the rate of fertility recovered significantly to almost the normal levels. Thus all the extracts possess reversible antifertility effects without any apparent toxic side effects.

The above data clearly elucidates that the oral treatment with papaya seed extracts, viz., benzene, butanol, methanol and benzene:butanol:methanol manifested reversible antifertility effects. The extracts were non-estrogenic, had no effect on hypothalamohypophysial gonadal axis and were non-toxic. Hence functional sterility
could be induced in both rats and mice by the papaya seed extract treatments.

The above findings are significant contribution in the field of contraceptive development from plant products.

Based on the above data, further studies in identifying the active component(s) of the various extracts are underway.