CHAPTER IV

DISCUSSION

PART I

A total of 175 females with clinical complaints of menstrual anomalies were screened for endocrine, haematological and biochemical alterations if any. In these patients cytogenetic studies were also carried out. The patients were further grouped as follows;

I Primary amenorrhea
II Secondary amenorrhea
III Oligomenorrhea
IV Primary sterility
V Secondary Sterility
IV Females with various cytogenetic abnormalities.

Among these 175 females, 88 cases were referred with complaints of primary amenorrhea, 27 females had secondary amenorrhea and 21 cases were reported as cases of Oligomenorrhea. Moreover, 18 and 14 females had primary and secondary sterility respectively. Another six cases were found to have
cytogenetic abnormalities or delayed puberty.

PRIMARY AMENORRHEA

Primary amenorrhea was found among relatively high percentage of females in this study with normal karyotype (46,XX). Hence, amenorrhea in these patients was due to endocrine imbalance which could be correlated with gynatresia. Geisthoval et al. (1983) have also correlated detailed ovarian morphologic characteristics with endocrine parameters and emphasised the significance of monitoring ovarian morphology by ultrasound scanning in patients with menstrual disorders which is in confirmation with our findings. Moreover, Coulam (1982) has reported that errors in gonadal development can cause amenorrhea in females.

In the present study, elevated gonadotropin levels with low E₂ was most commonly observed among these cases and could be correlated with poor development of the gonads and internal genitalia, i.e. absence of ovaries/uterus and small ovaries/uterus. These findings suggested that the females had primary ovarian failure, which was the major cause of amenorrhea. In a similar study of 182 patients, Coulam (1982) reported 81 cases of primary amenorrhea or primary ovarian failure exemplified by elevated gonadotropins with low levels of serum estradiol. The same author has also observed that ovaries had undergone differentiation but their function failed before the usual expected time of menopause. Moreover, in our laboratory also it was reported earlier that primary amenorrhea was mainly related with primary ovarian failure (Highland, 1989; Pandya, 1994) which is supporting our present findings.
Furthermore, high levels of prolactin were obtained in certain cases of primary amenorrhea. Other workers (Moghissi and Wallach, 1983; Monroe et al., 1981; Speroff et al., 1989) have reported that elevation of prolactin hormone is a common cause of amenorrhea and this elevation in prolactin appears to be due to inhibition of pulsatile secretion of GnRH resulting in amenorrhea. However, in the present study, with elevation of prolactin, gonadotropins were also observed to be on the higher side which is contradictory with previous observation. This condition could be attributed to physiological stress rather than pathological condition, since emotional states and a variety of physical stresses could induce PRL release (Modell et al., 1990).

McDonough (1978) has described the relationship between 46,XX females with primary ovarian failure and such patients are called as chromosomally competent ovarian failure (CCOP). The presence of normal genetic complement and karyotype ensure normal stature, development of secondary sex characters and normal distribution of pubic as well as axillary hair. However, in the present study, 15 cases had normal karyotype with underdeveloped uterus but normal ovaries. These patients had low gonadotropins and estradiol suggesting secondary ovarian failure. The secondary ovarian insufficiency indicates hypofunction of hypothalamo-pituitary-gonadal axis. The ovarian insufficiency may be due to the suppression of secretion below its critical range (Speroff et al., 1989) and which was classified by these authors as hypothalamic amenorrhea which is similar to our present findings.

Seven cases of primary amenorrhea group showed normal development of
gonads and hormonal profile. According to Alper and Garner (1985) and McDonough (1978) amenorrhea is not only dependent on hormonal or cytogenetic abnormalities, but it may also be dependent on immunological factors. In the present study also, amenorrhea in these females could be due to immunological problems leading to infertility or amenorrhea. This aspect needs further detailed investigation.

SECONDARY AMENORRHEA

Twenty seven cases were found to have a history of cessation of menstruation 2-6 years after menarche. These patients also had normal karyotype (46,XX) which suggested that amenorrhea is more frequently caused due to endocrine factors or abnormal gonadal development/differentiation. Among these cases, high gonadotropins with low estradiol levels were found in 9 cases with normal gonads and internal genitalia suggesting premature ovarian failure. In the 8 cases with abnormal development of gonads and uterus also, the gonadotropin levels were elevated with low estradiol. According to Givens (1986) the deficient ovarian function or ovarian failure is due to disorders within the gonads as manifested by lack of response of ovary to gonadotropin stimulation. These results in deficient follicular production and low estradiol levels. Moreover, similar results was obtained by Highland (1989) and Pandya (1994) which are in support of our findings.

The increased levels of LH with normal FSH and $E_2$, resulting in an
elevated LH/FSH ratio as observed in 4 cases of secondary amenorrhea suggest the possibility of hypofunction of ovaries due to cyst formation. This was confirmed by ultrasound scanning in these 4 cases of secondary amenorrhea. Berger et al. (1975) and Vejisted and Albrechten (1976) have classified the cases of polycystic ovaries into those with elevated LH levels. The cases investigated here, had high serum LH and altered LH/FSH ratio. Givens (1986) reported that most polycystic ovaries contained an increased number of atretic follicles in all stages of atresia. The persistence of the atretic follicles with the theca cell hyperplasia is due to stimulating effect of increased LH levels which could be in the range of midcycle surge or equivalent to post menopausal values. Early evidence indicated that the elevated LH levels were due to an increased sensitivity of pituitary to stimulation manifested primarily by an increase in LH pulse amplitude rather than pulse frequency (Kazer et al., 1987).

Persistently elevated LH level and LH/FSH ratios, with chronic anovulation related to presence of ovarian cysts have been demonstrated by several workers (Baird et al., 1977; Yen, 1980; Cave and Strek, 1983; Goldzicher and Dizerga, 1985; Moitz et al., 1985). However, they failed to identify a specific hormonal pattern with polycystic ovaries while in the present investigation, high LH with normal FSH were evident. Hirsuitism and obesity were however not evident in the patients found to have polycystic ovaries in the present study as reported by Stein and Leventhal (1935).

The PCO manifested only endocrine alterations and no chromosomal anomalies. On the other hand, several investigators (Cooper et al., 1968; Givens
et al., 1971) have correlated cytogenetic abnormalities with this disorder.

Six cases of secondary amenorrhea had normal development of gonads, endocrine profile as well as normal karyotype suggesting no detectable cause of amenorrhea in these patients.

**OLIGOMENORRHEA**

Twenty one cases complained of irregular menstruation (Oligomenorrhea) with normal karyotype, secondary sex characters as well as normal development of internal genitalia.

Out of 21, 11 of the oligomenorrheic patients had elevated levels of prolactin and gonadotropins with low serum estradiol. According to Speroff et al. (1989) a low estrogen environment associated with amenorrhea prevents a normal response to prolactin. Moreover, stress could induce alterations in prolactin secretion (as described earlier) which causes irregularities in menstruation. According to Fries et al. (1974) mental pressure, trauma, social stigma can also induce irregularities or amenorrhea in females. In our study, the secondary sex characters of oligomenorrheic cases were well developed with no clinical manifestations of hirsuitism. These observations confirm with those of Siegberg et al. (1986) who also described causes of oligomenorrhea with no signs of hirsuitism or obesity, normal female type breast development and secondary sex characters with no obvious cause of increased mean length of cyclicity. Moreover, Reiter et al. (1974) reported that menstrual irregularities are the result of an immaturity of estrogen induced positive feedback. This may be the reason for
irregularity in menses in the patients investigated in this study.

In the same group, 7 patients had low levels of gonadotropins and estradiol. According to Coutts et al. (1988) oligomenorrhea was observed in 21% of females with low gonadotropins and luteal phase defect. Coutts and his associates concluded that oligomenorrhea is a condition which is subjected to variation. The results of the present study also revealed wide variations in the hormone pattern of cases of oligomenorrhea which were similar to the observations of Coutts et al. (1988).

PRIMARY STERILITY

Primary sterility with anovulatory inability to conceive was commonly found among several women married for a period of 3-12 years. These individuals had well developed secondary sex characters as well as normal karyotype. In some cases with suspected anovulation, the ovulation was monitored periodically by ultrasound scanning.

The occurrence of primary sterility has been attributed to several diverse factors. One of them being ovulatory disorders (Kliger, 1984; Brown et al., 1987). Breckwoldt et al. (1986) estimated that approximately 30-40% of female infertility was due to ovarian dysfunction. In the present study, low levels of serum gonadotropins and E₂ but with elevation of prolactin was found in 8 cases. Infertility due to hyperprolactinemia has been described by Speroff et al. (1989) where high prolactin levels interfere with synthesis of GnRH as well as steroids. Bohnet et al. (1976) have also reported that elevated PRL is responsible for
impairing GnRH as well as ovarian steroid production. The findings in the present study are in agreement with the above observations.

Three patients having primary sterility had elevated levels of FSH. This is often attributed to onset of premature menopause. During the perimenopausal period, it is normal for FSH levels to begin to rise even before bleeding has ceased (Shearman and Korenman, 1975). This is true whether the perimenopausal period is premature at age of 25-35 or at the normal time. It is believed that FSH is partly under the negative feedback control of inhibin, a peptide produced by granulosa cells. The production of this peptide may be inadequate and the patient may have elevated levels of FSH despite continued cyclicity. Same observation was made in the present study.

Seven females of this group had no detectable changes in hormonal profile. McBain and Pepperell (1987) have suggested that in such cases, several tests have been performed and repeated but no abnormalities could be detected. Hence, such patients were categorised as having idiopathic infertility. Moreover, Highland (1989) and Pandya (1994) from our laboratory reported that 59% and 31% of females respectively had complaints of unexplained infertility. The causes for such unexplained infertility may be difficult to determine.

SECONDARY STERILITY

Fourteen patients were referred with history of secondary sterility but were found to have normal karyotype, secondary sex characters as well as normal development of internal genitalia.
Two patients of this group had normal prolactin, E$_2$ and FSH with elevation in LH levels. According to Speroff et al. (1989) abnormal pattern of LH secretion viz. elevated or decreased levels of LH may be associated with an inadequate luteal phase which could be the main cause of sterility in these females.

Other 5 cases showed low levels of E$_2$. Failure of ovulation can be associated with lower levels of E$_2$ which could be correlated with luteinized unruptured follicle in infertile females (Janssen Caspers et al., 1986) which is in confirmation with our present findings.

**MISCELLANEOUS**

**46,XY PHENOTYPIC FEMALE**

Phenotypic females with male genotype have been described by several authors (De Whurst, 1971; Naftolin and Judd, 1973; Bardin et al., 1973; Khodr, 1979; Medina et al., 1980). The patients have XY sex chromosomal constitution but with well formed breast and female phenotype, absent or scant sexual hair, normal appearance of vulva with short blind vagina without uterus or cervix.

The two patients investigated in this study, were phenotypically female and reared as such but had a male genotype (46,XY). Case No. 979 showed levels of FSH, LH, T, E$_2$ and PRL which were comparable to normal males, compatible with their genotype but inconsistent with the development of female secondary sex characters. Judd et al. (1972) and Boyer et al. (1978) have also reported 46,XY females with hormone profile comparable to normal males. In case No. 1205 however, the serum levels of FSH and LH were elevated with low E$_2$ suggesting
primary gonadal failure with testosterone comparable to that of normal males. Ultrasound scanning reports revealed the presence of undescended testis in these cases, which therefore, explains why the serum testosterone levels are comparable to that of normal males. The androgen production from the testis of such individuals has been observed to be normal or near normal. The defect probably arises due to impaired tissue utilization of the hormone. It is also known that these androgen insensitivity syndromes have varying phenotypic characters (Cundy, 1986). These cases are phenotypically female and mistakenly reared as such. In the presence of androgen but absence of functional androgen receptors, Mullerian duct regression occurs, with improper differentiation of the internal and external genitalia (Hurley and Burger, 1984). The presence of estrogen and absence of testosterone action causes a female phenotype to develop.

Two females of this study were probably having androgen insensitivity syndrome of the complete type and hence reared as females. Moreover, these patients lacked Mullerian duct, except a blind vagina showing an otherwise functional testis capable of bringing about Mullerian regression.

**TURNER SYNDROME**

Turner Syndrome is a well known chromosomal disorder, occurring in 1:2500 female births (Ferguson-Smith, 1965) and is associated generally with the absence of one X chromosome (45,XO) (Ford et al., 1959). A variety of X chromosomal abnormalities and mosaicisms are now known to be associated with this syndrome with specific cogenital malformations viz., short stature, shield-
shaped chest, cubitus valgus, ptergium colli, hypoplastic nails, low hair line, collectively termed as Turner Stigmata.

Turner characteristics were observed in one girl of 15 years old diagnosed as probable Turner Syndrome. A parallel cytogenetic study carried out confirmed 45,X cell line of true Turner karyotype. In a patient with 45,X karyotype, case No.1283 had features of the syndrome viz., short stature, ptergium colli, low hair line, shield shaped chest, etc.

The findings of significantly elevated gonadotropin levels with subnormal levels of estradiol indicate primary ovarian failure and is correlated with ultrasonography which showed absence of ovaries and uterus. Givens (1986) and Villadollid et al. (1986) have noted that elevated value of FSH (<40.0 mIU/ml) and LH (<25.0 mIU/ml) in association with primary amenorrhea and Turner syndrome features establish the diagnosis of hypogonadal function due to ovarian failure. Ovarian development, including germ cell migration may occur in the presence of a single X chromosome, but this may be followed by disappearance of follicles (Sheaman, 1985a). Impaired ovarian differentiation results in a hypofunctional gonad, which shows poor response to gonadotropin stimulation and low secretory activity resulting in hormonal imbalance which has a bearing on phenotypic and pubertal development.

McDonough et al. (1977) and Millet et al. (1985) have reported both mosaic and non-mosaic Turner patients with elevated gonadotropin and low E₂ indicating a higher frequency of ovarian failure, than observed in our study. McDonough et al. (1977) have reported as many as 52 out of 82 amenorrhea cases as having
variant or Turner karyotype. Although it has been reported that 10% of non-mosaic and 20% mosaic Turner patients will have normal menses (King et al., 1978; Jacquemyn et al., 1989), they had not experienced menarche but showed clear indication of absence of both ovaries and uterus.

**PSEUDOHERMAPHRODITE**

Case No.1304 assessed in this investigation was found to have a female genotype (46,XX) but with well developed male secondary sex characters with normal female type hair distribution as well as behaviour. External genitalia was male type with well developed labia majora, Labiminora and the vagina were found to be absent in the patient. Ultrasound scanning revealed that there was normal development of ovaries as well as uterus and cervical canal. However, testis was found to be absent in this case. According to Shearman (1985b) these individuals are female at the chromosomal, gonadal and internal genital level. At birth however, the external genital sex resembled the sex seen in common forms of congenital adrenal hyperplasia. This condition is related to exposure of an otherwise normal female fetus to an abnormal androgenic stimulus in uterus possibly secreted by the hypoplastic adrenal cortex. These androgens cause virilization of the external genitalia in the genetic female fetus and female pseudo hermaphroditism (Levine and New, 1986). The hormonal profile of case No. 1304 showed normal gonadotropin levels with low E$_2$. Testosterone was found comparable to normal female. In the case No. 1304 investigated in the present study, endocrine findings indicated an imbalance in the T/E$_2$, suggesting increased
androgen action in this case.

**DELAYED PUBERTY**

Adolescent growth spurt results from synergism between gonadal sex steroids and growth hormones as long as other endocrine functions are normal (Brook, 1982). The lack of onset of pubertal characters (viz. breast development, axillary and pubic hair) by the age of 13 or lack of menarche by age of 15, represents a significant delay in development of puberty. Delayed puberty was not found to occur commonly among females in this region (Gujarat and its vicinity) as only 2 adolescents in a selected group of patients, were found to have absence of sexual maturation at puberty. These girls were 16 years of age. Puberty in Indian girls has been shown to commence at a mean age of 11.08 years (Prabhakara et al., 1972).

Depending upon the serum gonadotropin levels, Lee (1986) has categorised delayed puberty as hypogonadotropic or hypergonadotropic conditions. In this investigation, girls presenting with delayed onset of puberty appeared to fall in the former category, as evidenced by the findings of significantly low serum levels of FSH, LH and E2. Features of marked dwarfism, mental retardation, hypotonia, obesity, thyroid or adrenal disorder were not observed. The clinical examination suggested therefore that these were cases of constitutional delay in activation of the hypothalamic-pituitary-gonadotropic axis, causing a prolonged pubertal state. Persistence of puberty in the form of hypogonadotropic-hypogonadism was also reported by other workers (Brook, 1982; Santore et al., 1986).
In a survey carried out on Indian girls, Vaidya et al. (1986) have shown clinical features and endocrine findings comparable to the present observations, confirming constitutional pubertal delay as a common cause of the disorders. The two patients investigated in this study, were from low socio-economic class. According to Speroff et al. (1989) poor nutrition (anorexia nervosa, chronic illness, renal disease) may also lead to hypogonadotropic delayed growth and development.

BIOCHEMICAL EVALUATION

There were no alterations in levels of haemoglobin, blood glucose and serum cholesterol in all groups of females except the oligomenorrhea group. These findings suggest that basal metabolism is not affected in females with infertility. Same observations were made in our laboratory with no alterations in haemoglobin level, blood glucose and serum cholesterol in females with complaints of infertility (Chinoy and Joshi, unpublished observation).

In the group of oligomenorrheic females, the blood sugar and serum cholesterol were lower than normal suggesting mild alteration in basal metabolism in relation to irregular cycle.

Therefore, from the obtained results, in the present study, amenorrhea was the most common cause for infertility. The primary ovarian failure was the main cause among amenorrheic group of females. Secondary and oligomenorrhea were also related to hormonal imbalance in these females. However, the primary and secondary sterility group had unexplained infertility or idiopathic infertility which could be related with stress in these females. Chromosomal abnormalities
associated with these disorders was found to be very rare in Ahmedabad city and its vicinity. Hence, in the present study, hormonal imbalance seems to be more prevalent in infertile females.
PART II

The effects of administration of a benzene extract and an alcoholic extract from ripe papaya seeds at a dose of 20 mg/Kg body weight/animal/day for 30 days were studied in female adult rats in order to investigate the efficacy of both the extracts on reproductive organs, cyclicity, fertility rate and evaluation of toxic effects of the extracts, if any. The dose was administered for 30 days and animals were autopsied on day 31st.

The extracts was administered orally to animals every day for 30 days. In our laboratory various routes for administration of the extracts i.e. intramuscular, subcutaneous, gastric intubation have been tried on both sexes of rats, guinea pigs and mice (Chinoy and Trivedi, 1983; Chinoy and Geetha Ranga, 1984; Chinoy and Sam George, 1983, Chinoy et al., 1984/1985) and all routes of administration were found to be effective in both males as well as on females for inducing infertility. Thus, in the present study, the oral route was selected for the treatment which is comparably an easy way to administer the extract and also more feasible.

The parameters studied for assessing the anti-fertility effects of benzene as well as alcoholic extracts of papaya seeds were i.e. body weights, weights of reproductive organs, estrous cycle, fertility rate, structure and metabolism of reproductive organs and some selected toxicological parameters. The WHO MB-70/71 protocol was employed to investigate whether the extracts possessed any estrogenic effects or otherwise. The possible reversibility of the induced effects were investigated by withdrawing the treatment for 1 month after administration
of extracts for a specific period.

The results revealed that the body weight of all treated groups did not show any significant changes after treatment. This suggested that the extracts do not promote weight gain causing obesity and/or water or electrolyte retention. Similar result was also obtained by Das (1980) in rats by treatment with papaya seed extract. Moreover, the earlier work from our laboratory has also elucidated that during administration of different papaya seed extracts in female as well as male rats, the body weight was not affected (Chinoy and Geetha Ranga, 1984; Chinoy et al., 1984/1985; 1994b). Similarly, the ovarian weights were not changed throughout the treatment by both the papaya seed extract treatments. The histological studies showed atretic primary and secondary follicles as well as Graafian follicles in the ovary and changes in corpus luteum number as well as alteration in its diameter in treated animals. The same observations were also recorded by Chinoy and Trivedi (unpublished observation) in our laboratory.

The concentration of cholesterol and enzyme activities of 3β hydroxy steroid dehydrogenase (3β HSD) and 17β hydroxy steroid dehydrogenase (17β HSD) were not affected in the ovary by both extract treatments which suggested that the extracts did not alter ovarian steroidogenesis in treated female rats. In case of male rats also the extract did not affect the levels of cholesterol in testis and serum testosterone (Chinoy and Geetha Ranga, 1984; Chinoy et al., 1984/85). Our present findings also revealed that the extracts did not affect pituitary hormones, FSH and LH similar to the data in male rodents. The serum cholesterol and circulating estrogen levels were also not altered in all groups of animals in the
present study. Thus, it is evident that steroidogenesis proceeds unhampered in the treated animals. The data also reveals that the hypothalamo-pituitary-gonadal axis is not disturbed by the extract treatment in females as was reported earlier in male rodents (Chinoy et al., 1984/85; Chinoy and Geetha Ranga, 1984). This finding is significant since the libido will not be affected and the extracts do not seem to possess any antigonadotrophic effects.

It is known that RNA synthesis or activity of RNA polymerase in reproductive organs are hormone dependent (William-Ashman et al., 1964). The higher level of RNA in uterus of treated animals observed in our study could be related to the histological changes therein. In uterus, the alteration in RNA level could also be due to blockage in protein synthesis in treated animals. In the present study, the extract ingestion revealed a significant decline in DNA in uterus which could also be correlated with the decrease in total uterine protein by both extracts of papaya seed and alterations in myometrium, endometrium and lack of glands. The above mentioned changes in uterus after treatment might influence its internal milieu, growth of glands and hence secretion, as is evident from the decrease in glycogen levels and alteration in phosphorylase activity. However, the seed extracts did not affect the sister chromatid exchange or cause chromosome aberrations in human leucocytes in vitro (Chinoy et al., unpublished observation).

These changes might not be conducive for nidation or implantation of the fertilized ovum in the uterus of female rats. It is known that the mechanism of egg implantation requires proper interaction between progesterone and estrogen including a series of irreversible modifications (Psychoyos, 1966; Mayer, 1966) and
a slight disturbance in this hormonal balance may lead to the instability of pregnancy. Progesterone independently or together with estrogen inhibits the release of LH and blocks ovulation in rats (Watnick, 1964). Moreover, it was reported (Psychoyos, 1966; Nutting and Sollman, 1967) that low doses of progesterone could inhibit or delay implantation in female rats. Prakash (1978) and Prakash and Mathur (1977) suggested that the anti-implantation activity of leaf extract of Artobotrys odoratissimus Linn is due to its anti-estrogenic mode of action which could work in three ways: (a) may produce a non receptive stage of uterus where implantation will not occur, (b) delay the implantation of egg or (c) have direct blastotoxic action. It is likely that in the present study too, the extracts manifested similar effects. According to Farnsworth (1975), the principle responsible for anti-implantation effects of papaya seed might be 5 hydroxy tryptamine, but it remains to be seen if only this compound or others too are involved in the process and Farnsworth’s data needs to be confirmed.

The lack of implantation might be related to irregularity in cyclicity with predominance stage of diestrous. The disruption of cyclicity has been associated with hypothalamic inhibition (Farnsworth et al., 1975) followed by the probable disruption of gonadal axis. On the contrary, in our earlier study (Chinoy et al., 1994b) and present work, no effects on pituitary gonadal axis were evident. Hence it is likely that the extracts which themselves are non-estrogenic (as tested by WHO protocol MB 70/71) do possess some antiestrogenic influence. This aspect needs to be studied in considerable details in future. It was reported earlier (Chinoy et al., 1994b) that the extract manifested abortifacent effect in female rats.
This was due to the enhancement in contraction of uterus of treated rats when subjected to different doses of oxytocin *in vitro* as compared to the control (Chinoy et al., 1994b).

The above mentioned changes together with absence of implantation resulted in 100% negative fertility rate in the *Carica papaya* seed extract treated rats in corroboration of results obtained by Chinoy et al. (1994b). Moreover, Garg et al. (1970) and Sareen et al. (1961) also reported that antifertility effect of papaya seed in female rat and mice respectively were due to its anti-implantation activity.

The mechanism of action of *Carica papaya* seed extracts (benzene and alcoholic) seems to be antiestrogenic at dose of 20 mg/Kg body weight/day/animal. The metabolic effects and those on the histology of ovary and uterus, fertility rate, estrus cycle suggest that the extracts have a direct action on the gonadal structure and functions.

The toxicological studies showed that histology of liver and kidney as well as their metabolism were by and large not affected in treated groups of animals. However, liver glycogen and phosphorylase activity in treated female rats indicated insignificant alterations. The serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels were within the normal range even after extract treatment. Hence it is evident from the present data that *Carica papaya* seed extracts did not alter liver function in treated rats. Earlier studies carried out in our laboratory by Chinoy et al. (1994c) on male mice as well as rats and guinea pig (Chinoy et al., 1984/85; Chinoy et al. unpublished observation) respectively also showed similar results in treated groups. The LD50
dose was 15 gm/Kg body weight (Chinoy et al., 1994c) which is much higher than LD50 dose of 5 gm/Kg body weight recommended by the WHO as of non toxic nature for a plant product. This revealed that both the extracts were non toxic.

The effect of withdrawal of the treatment for one month was investigated to study the reversibility of the induced effects if any. From the results it is evident that recovery in most of the parameters occurred by the withdrawal treatment which also restored the cyclicity and 100% positive fertility rate in withdrawal groups of animals.

From the above data, it is evident that the oral treatment with benzene and alcoholic extracts of papaya seeds manifested reversible anti-estrogenic, anti-implantation and anti-fertility effects by 30 days. The extracts were non estrogenic, had no effect on hypothalamic-hypophysial gonadal axis and were nontoxic. Hence functional sterility could be induced in female rats by the treatment with benzene as well as alcoholic extracts of *Carica papaya* seeds. This is a significant lead in the field of female contraception.