DISCUSSION
Discussion

Contraceptive development in a natural way by exploiting the ancient idea of using antifertility plant’s, especially for males, has been subjected to scientific evaluation in recent years. The major advantages of using drugs of plant origin are that they are cost-effective and have the least side-effects (Chaudhury, 1985). The major target for task force on plants for fertility regulation of WHO is to identify the novel drug prototypes found in plants which have been reported to have fertility regulation properties. Compounds that are being sought in particular are those that are orally active, non- steroidal, non-estrogenic, safe and effective for the prevention or disruption of implantation in women and those that inhibit spermatogenesis or interfere with sperm maturation in men (Griffin, 1988).

Numerous herbs have been used historically to reduce fertility and modern scientific research has confirmed antifertility effects in some of the herbs tested. Herbal contraception offers alternatives for contraceptive options or who just want to try different ways and in reducing the fertility, it would be better than no birth control at all. There’s a lack of information on using herbs for contraception, but there are historical references with clues to what ancient women did, and the scientific community has published some studies, mostly on animals, showing that some of the herbs do seem to have contraceptive effects. There have also been informal studies where the herbs were tested by women for contraception.

Oral herbal contraceptive would allow couples control their fertility without consulting a health worker, which in turn would markedly increase the number of couples practicing family planning. Other advantages of such a contraceptive would include the familiarity rural people have with herbal medicines, the fewer side effects associated with herbal preparations, their ready availability from local sources, and protection of privacy. There are many references to
plants in India with antifertility properties. Since 1966, the Indian Council of Medical Research (ICMR) has been conducting research to identify a herbal contraceptive, as have the other organizations. Plants that have exhibited antifertility activity in clinical trials include *Hibiscus rosasinensis* (benzene extract of the flower petals suppresses implantation); *Rudrapushpaka* (extract of the flower petals prevents pregnancy); *Embelia ribes* (pregnancy prevention); *Davcus carota, Butea monosperma,* and *Sapindus trifoliatis* (seeds have anti-implantation effect); and *Mentha arvensis* (leaves have antiimplantation effect). The Central Drug Research Institute in Lucknow, India, in collaboration with the US National Institutes of Health, the World Health Organization, and the ICMR confirm anti-implantation activity in *Ferula jaeschkeana, Bupleurum marginatum, Lepidium capitatum, Caesalpinia sepiaria, Lonicera japonica, Juniperus communis, Lotus corniculatus, Lamium allum,* and *Acacia farnesiana.* In China, scientists have evaluated the cotton seed extract gossypol as a male contraceptive. They are now studying the possible antifertility effect on men of the plant *Tripterygium wilfordii.* From all the aforementioned plants as well as others under investigation, three possible types of contraceptives could be developed: an anti ovulatory contraceptive; a postcoital contraceptive; and male contraceptive. Some obstacles to their development include difficulties in obtaining adequate quantities of the herbs, a shortage of clinical pharmacologists and clinicians interested in conducting clinical trials, and lack of long term financial support (Chaudhury, 1980).

The search for plants for male fertility regulation is comparatively smaller as it is directed towards the inhibition of millions of sperms produced daily as against one ovum released every month in females. However, attention has been given in this modern era and attempts have been made to bring out safe, effective plant preparations as ideal contraceptives for males (Zeherea et al., 1998).
In the present investigations, it was observed that the Guar pea treatment did not cause significant reduction in the animal body weight when compared to control. This shows the absence of toxic side effect of the plant in the animals tested. The same result has been found in the administration of *Alstonia scholaris* bark extract (Gupta *et al*., 2002), *Strychnos potatorum* seed extract (Gupta *et al*., 2006), *Tuniperus phoenica* (Shkukani *et al*., 2007) to male rats and *Achillea millefolium* flower extract (Montanari *et al*., 1998 a) to male mice did not cause any significant change in the body weight of treated animals when compared to the control groups.

In some other studies like the administration of N-butyldeoxynojirimycin (NB-DN) at higher doses of 600 mg/ Kg reduces the body weight of mice (Platt *et al*., 1994) than in low doses of 15-150 mg/Kg. In another study, it was found that still higher dosage of 1200-2400 mg/Kg of N-butyl deoxynojirimyein (NB-DNJ) significantly reduced the body weight of orally treated male mice (Spoel *et al*., 2002). Udoh *et al*., (1992) also found a decrease in the body weight of male Wistar rats after the administration of gossypol acetate.

In the present investigations, it was observed that there was significant reduction in the weight of the testis and other reproductive organs. Similar result has been found in Oral administration of saponins of *Albizzia lebbeck* bark for 60 days. It brought about a significant loss in testis weight, which is known to be mostly related to number of spermatids and spermatozoa present in the tissue. (Gupta *et al*., 2005). The significant reduction in the weight of reproductive organs indirectly supports the reduced availability of androgen (Zeherea *et al*., 1992). Androgen deprivation not only suppresses spermatogenesis, leading to low sperm concentration, but also alters the epididymal milieu which renders it hostile for maturation and survival of the spermatozoa (Setty, 1979 & Rao, 1988). Different doses of extract containing flavonoids from the seeds of *V.negundo* have shown antifertility effect in male rats (Das *et al*.,
2004). In the same way, C.psorolloids has flavins (Dorothy et al., 2006) and has affected the testis and accessory reproductive organs. This reduction may be due to the decreased production of seminiferous tubular fluid, which contributes to the weight of the testis (Ghosh et al., 1993).

Other research workers have reported that the oral administration of alcohol extract of Sapindus emarginatus, Terminalia belerica (fruits), Cuminum cyminum and Allium cepa (seeds) (Venkatesh et al., 2002) and Crotalaria juncea seeds (Vijay Kumar et al., 2004) brought about a decrease in the weight of reproductive organs indicating that the circulating testosterone levels caused this loss in the weights of organs. Momordica Charantia seeds showed the antispermatogenic activity by testicular weight reduction and inhibition of spermatogenesis (Naseem et al., 1998). The reduction in the testicular weight may be attributed to the altered production of seminiferous fluid (Ghosh et al., 1993). Significant changes in testicular and epididymis weights were found when etropine was administered orally to male mice and rats (Malik et al., 1995). The same result was found when the aqueous extract of Stephania hernandifolia (Ghosh et al., 2002) and ethanol extract of Piper betle (Sarkar et al., 2000) on testicular, epididymal, seminal vesicle and ventral prostate weights in male albino rats and mice due to low plasma level of testosterone (Bortlett et al., 1990 and Ghosh et al., 1983). Another study showed significant reduction in the weight of testis, epididymis and seminal vesicle in male rats treated with benzene extract of Ocimum sanctum leaves (Ahmed et al., 2008). There was also significant reduction in the weight of reproductive organs in Albizia lebbeck (Gupta et al., 2005) and Ruta graveolens (Khouri and Ei-Akawei, 2005) treated male albino rats. Oral administration of lipidosterolic extract of Hyphaene thebaica for 45 days brought about a significant loss in testis weight (Hetta et al., 2005).
Testosterone, an important androgen, plays a pivotal role in maturation, spermatogenesis and the maintenance of accessory sex organs (Keel & Abney, 1981). The structural and functional integrity of reproductive tissues depends on the circulating androgen (Chinoy et al., 1982) and therefore, any small change in testosterone content may result in reductions in the weights of the reproductive organs. The ability of the extract fed mice to mate might have been possible because of low androgen concentration (Sondersten, 1979) or owing to circulating plasma testosterone, which was sufficient for normal mating behaviour, but insufficient for the maintenance of fertilizing ability of the epididymal spermatozoa (Bhasin et al., 1988). Androgen deprivation not only suppresses spermatogenesis, leading to low sperm concentration, but alters the epididymal milieu also, which renders it hostile for physiological maturation and survival of the spermatozoa (Setty, 1979; Rao and Mathur, 1988; Rao and Shah, 1988). Studies with Embelia ribes berries have been shown to reduce circulatory testosterone levels and secretory activity of the accessory organs resulting in the reduction in the volume of semen in male Bonnet Macaques (Purandhare et al. 1979). Similar result have been reported in that testicular weight reduction accompanied by decreased serum testosterone levels in male rats treated with the extracts of Quassia amara (Raji and Bolarinwa, 1997), Azadirachata indica (Raji et al.,2003) and gossypol, a phenolic compound extract of the cotton seed ( Qian and Wang, 1984). Since testis and other accessory reproductive organs are androgen dependent, they serve as indicators of the Leydig cell function or androgen action (Rommerts, 1988). The accessory male reproductive ducts and glands are morphologically and physiologically depend on the production of androgen (Williams- Ashman & Reddy, 1972). In same manner, C.psoralioids treatment we find significant decrease in the circulatory testosterone levels and significant reduction of sperm. From the above, we can conclude that C.psoralioids has antispermatogenic activity.
Histometric studies indicate the effect of ethanolic extract of *Cyamopsis psoralioides* pod cause significant reduction in the diameter of seminiferous tubules and Leydig cells in group II, III and IV animals. Similar results have been found in leaf extract of *Stephania hernandifolia* treated rats (Ghosh *et al*., 2002), which also supports the low plasma level of testosterone (Parvinene, 1982). The administration of Brahmi in mice (Singh and Singh, 2009) and *Ocimum sanctum* in rats (Ahmed *et al*., 2008) have revealed significant regression in the seminiferous tubular diameter, with marked degenerative changes in the tubules. The reduced testicular weights and shrunken seminiferous tubular dimensions indicate wide spread damage (Keel and Abney, 1980). When *Opuntia dillenii* (Gupta *et al*., 2002) and *Colebrookia oppositifolia* (Gupta *et al*., 2001) extracts treated to male rats, showed significant reduction in seminiferous tubule diameter after treatment. Oral administration of saponins of *Albizzia lebbeck* bark for 60 days brought about a highly significant shrinkage in the seminiferous tubule diameter of testis (Gupta *et al*., 2005). The etoprine administration of saponins of *Albizzia lebbeck* bark for 60 days brought about a highly significant shrinkage in the seminiferous tubule diameters (Malik *et al*., 1995). Since the leydig cells form the major endocrine portion of the gonads. They produce male androgens including testosterone which is controlled by the anterior pituitary gonadotrophins (Follicle stimulating hormone/Luteinizing hormone/ interstitial cell stimulating hormone, FSH/LH/ICSH). This disruptive effect could be direct or indirect effect of androgen on the tubules. Since the tubules require a high concentration of androgen for cell maturation and function, the disruption of the tubules could lead to a lower concentration of androgen and this could cause the histological changes observed in the tubules (Sharma and Jacob, 2001). Seminiferous tubules make up about 90% of the normal testis and the testicular weight loss in higher dose groups may be attributed to the spermatogenic disruption and degeneration of
germinal elements (Kantak et al., 1992). The reduced testicular weights and shrunken seminiferous tubular dimensions which results in the increase in space between the tubules have become more in treated group. (Keel and Abney, 1980). The total volume of Leydig cells is positively correlated with the onset of spermatogenesis. Therefore shrinkage in Leydig cells of treated mice suggests a pronounced reduction of fertility (Hochereau de Reviers and Terqui, 1984). Regression of Leydig cells diameter after the treatment suggests the antispermatogenic activity of Cyamopsis psoralioides in male mice.

Similar results are found when ethanol extracts of Juniperus phoenicacones (Shkukani et al., 2007), Echinops echinatus roots (Padashetty and Mishra, 2007) and Alstonia scholaris bark (Gupta et al., 2002) were administered to rats and ethanol extracts of Amalakyadi churna (Seetharam et al., 2003) administered to mice showed significant decrease in seminiferous tubule diameter when compared to controls.

When Brahmi was administered to male mice, there was significant increase in the percentage of affected seminiferous tubules in the testis with degenerative features like significant reduction in the height of germinal epithelium and diameter of the tubules, loosening of germinal epithelium and exfoliation of germ cells (Singh and Singh, 2009). In P mice the marked degenerative changes in the seminiferous tubules after the treatment with gossypol tetra acetic acid (Singh and Rath, 1990; 2008) and the treatment with the aqueous leaf extract of Azadirachata indica have caused degenerative changes in the seminiferous tubule (Mishra and Singh, 2005).

In the study of benzene extract of Ocimum sanctum leaves in albino rats regression and degenerative changes in the seminiferous tubules, significantly decreased number of leydig cells and their nuclear diameter reflecting the depletion of androgen level are found (Ahmed et al.,
2008). It is supported by decreased number of germinal cells like spermatocytes and spermatids since these changes are completely androgen dependent (Dym et al., 1979). The observations related to the unique nature of the changes in the seminiferous tubules similar to every plant product assayed so far in male antifertility studies Viz. *Carica papaya* (Lohiya and Goyal, 1992), *Euphorbia hernifolia* (Mali and Chaturvedi, 1994), *Momordica charantia* (Naseem et al., 1998), *Coeculus pendulus* (Verma and lall, 1999), methanol stem extract of *Sarcostema acidum voigt* (Verma et al., 2002), stem extract of *Tinospora cordofolia* (Wild) (Gupta and Sharma, 2003), ethanol extract of *Semecarpus anacardium* (Sharma et al., 2003), saponins isolated from *Albizzia lebeck* bark (Gupta et al., 2005).

In our present study In Group II and Group III animals showed fewer sperms in the lumen of the testis and epididymis. The increased intratubular space found between the epididymal tubules of mice, treated with higher dose (Vijaykumar et al., 2004) also reports the significant reduction of sperm density in the cauda epididymis after treating mice with *Crotalaria juncea* seed extracts. Reduction in sperm density in cauda epididymides may be due to changes in the androgen metabolism. The principal cells of epididymis synthesize proteins, which have important role in maturation of spermatozoa (Kasthuri et al., 1995). Also there was reduction in the number of normal mature spermatozoa with the presence of abnormalities of sperm head like Amorphous, Banana, Pin, and Hammerhead.

Various plants like *Vinca rosea* (Murugavel et al., 1989), *Solanum xanthocarpum* (Rao et al., 1988), *Banbusa arundinacea* (Kumari et al., 1989), *Ocimum sanctum* (Kantak et al., 1992), *Carica papaya* (Lohiya & Goyal, 1992) and *Spirulina plantensis* (Murugan et al., 1993) have been reported to posses antifertility activity. Treatment with above said plant materials could increase the amount of abnormal sperm (Ghosesawar et al., 2003). In the same manner treatment
with Guar pea decreases the sperm count and increase the abnormality of sperm which leads to decrease in the concentration of sperm in the treated animals. Inadequate concentrations of spermatozoa fail to penetrate the cervical mucus and thus fail to fertilize the ova (Setty, 1979 & Rao, 1988). In all the treated groups there was significant decrease in sperm count. A semen sample from an ejaculate containing more than 20% abnormal sperm is considered poorly fertile (Ghoresawar et al., 2003). Morphologically abnormal spermatozoa are diagnostic aids for infertility. One cause of infertility is a high incidence of abnormality (Amann et al., 1981). In many of the plant-based contraceptives, inhibition of male fertility after administration of natural substances inhibits sperm counts. For male contraception, it is not enough to stop spermatogenesis, but to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm (Nikkanen et al., 2000). Likewise, in this study, the ethanol extract of *Cyamopsis psoralioides* pod exhibited a marked reduction in sperm counts of cauda epididymis, which suggested the alternation in sperm production in the testis, as compared to controls. The decrease in sperm count and the high number of morphological abnormal sperm indicates interference with testicular spermatogenesis (Parveen et al., 2003). The reduction in the number of spermatozoa may be due to decreased testosterone level which intern reduces the weight of the testis and accessory reproductive organs (Adhikary et al., 1990). These sperm number depletion suggest alterations in sperm maturation also (Sarkar et al., 2000).

In accord with others works on *Juniperus phoenica* (Shkukani et al., 2007), *Echinops echinatus* roots (Padashetty and Mishra, 2007), *Strychnos potatorum* seeds (Gupta et al., 2006), *Ruta graveolens* and *Cannabis sativa* (Sailani and Moeini, 2007), *Vitex negundo* (Das et al., 2004), *Quassia amara* (Parveen et al., 2003), *Hyphaene thebaica* (Hetta et al., 2005), *Alstonia scholaris* (Gupta et al., 2002), *Albizia lebbeck* (Gupta et al., 2005), *Semecarpus anacardium*
fruits (Sharma et al., 2003), *Sarcostemma acidum* (Verma et al., 2002), *Opuntia dillenii* (Gupta et al., 2002), *Monida whitei* (Watcho et al., 2001) and *Morinda lucida* extract (Raji et al., 2005) in male rats and also *Piper betle* (Sarkar et al., 2000), *Amalakyadi churna* (Seetharam et al., 2003), *Crotalaria juncea* seeds (Vijaykumar et al., 2004), Brahmi (Singh and Singh, 2009) in male mice showed significant sperm count reduction after treatment. Reduced number of spermatozoa also found when mice were treated with tamoxifen citrate (Rai and Vijayalakshmi, 2001) and when mice and rats were treated with etoprine (Malik et al., 1995). Even in humans, sperm count was decreased, when treated with Tripterygium hypogtaucum (Qian et al., 1988).

This is also supported by the significant increase in the abnormal morphology of sperms with the administration of *Vitex negundo* seed extract (Das et al., 2004), *Morinda lucida* (Raji et al., 2005), Brahmi (Singh and Singh, 2009) and *Quassia amara* (Parveen et al., 2003) in rats. Tamoxifen citrate (Rai and Vijayalakshmi, 2001) and N-butyldeoxynojirimycin (Spoel et al., 2002) induced sperm shape abnormalities in mice.

The treatment of Monosodium glutamate (MSG) to the Wistar male rats has affected the sperm morphology and increased the abnormal sperm when compared to the control animals (Nayanatara et al., 2008). Treatment with the lead acetate has increased the sperm abnormalities in the Swiss mice. (Acharya et al., 2003). Administration of Brahmi to the male mouse has increased the number of abnormal sperm (Singh et al., 2009). Administration of seed extract of *Momordica charantia* to the male rats has induced increase in the abnormal sperms in the epididymis (Girini et al., 2005). The oral ingestion of Molybdenum has increased the abnormal spermatozoa in the epididymis. (Pandey and Singh, 2002). Administration of gossypol to the red deer has increased the sperm abnormalities (Gizejenski et al., 2008). The intragastric administration of *Morinda lucida* to the male rats has observed 70% of abnormalities and most
of the abnormality was curved tail (Raji et al., 1985). The oral administration of *Mentha arvensis* to the male albino mice has increased the sperm abnormalities and tails of most of the sperms in cauda epididymis appeared coiled (Sharma and Jacob, 2002). The administration of root extract of *Ricinus communis* (Linn) to the male rats has affected the normal sperm morphology (Sandhya kumary et al., 2003). The extract of *Cestrum parqui* has revealed a significant damage to sperm membrane in both head and acrosomal membranes in human semen samples (Souad et al., 2007). Treatment with the neem leaf extract has induced large number of headless spermatozoa in the epididymis in male Wistar rats (Parshad et al., 1997). In contradictory to the above result Interestingly, it was observed that sperm morphology remained unaltered in Swiss albino male mice treated with *Piper betel* Madhumita et al., (2000). The administration of *Mikania laevigata* syrup did not affect the structure of sperm (Carlos graca et al., 2007) in male Wistar rat.

Oral administration of ethanolic extract of *Cyamposis psoralioides* caused statistically significant decrease in protein, glycogen and ascorbic acid levels in testis, epididymis and vas deferens. The cholesterol level has decreased in testis and vas deferens in Group II, III and IV animals. But at the dose levels different durations were used in this study, there were dose dependent effects in protein, glycogen, cholesterol and ascorbic acid levels.

In our present study, protein level in testis was reduced significantly. The same result was found by Verma et al., (2002), Yakubu et al., (2008), Chauhan et al., (2007) and Gupta et al., (2000) who reported a significant decrease in the testicular protein concentrations, when rat and mice were treated with the stem extract of *Sarcostemma acidum, Fadogia agrestis, Aegle marmelos* and root extract of *Barleria prionotis* respectively.
Protein is involved in almost every physiological system and the total proteins run parallel to the growth and is sensitive to estrogen and androgen respectively (Watson et al., 1987). Jones (1977) has reported that protein level is directly correlated with the secretory activity of epididymis, which in turn depends on the androgen levels. So it is evident that testicular function would be altered by reduced protein content (Robaire and Hermo, 1988). The low levels of testicular protein are usually indicative of inhibition of spermatogenesis (Dixit and Bhargava, 1983). The reduced protein content may also be another reason, as the growth rate of any organ is proportional to its protein content, since evidently FSH stimulates the development of spermatogonia to spermatocytes and also maintains the spermatogenic process (Connel and Eik-Nes, 1968).

The significant reduction in the protein level of testis was reported when bark extracts of *Alstonia scholaris* (Gupta et al., 2004) were given. The significant reduction of testicular and epididymal protein has also been reported by Venkatesh et al., (2002) after the treatment of *Terminalia belerica* (fruits), *Curcumin cuminum* and *Allium cepa* (seeds) to male rats. Treatment with the petroleum ether, benzene and alcoholic extracts of *Momordica charantia* seeds to rats showed significant reduction in testicular protein, when given orally, but it was highly significant when given intraperitonially (Naseem et al., 1998). The reduced protein may be another reason for reduction in the weight of reproductive organs. Because the growth rate of an organ is proportional to its protein content (Sarkar et al., 2000).

The epididymal protein was reduced in the present study, Similar result has been found in other plant materials, including *Sarcostema acidum* (Verma et al., 2002) *Barleria prionitis* (Gupta et al., 2000) and *Semecarpus anacardium* (Sharma et al., 2003) in rats and *Mentha arvensis* in mice (Sharma and Jacob, 2001). The principal cells of epididymis synthesize
proteins, which have an important role in maturation of spermatozoa (Kasthuri et al., 1995). Alterations in the secretion and function of these proteins might have impaired sperm maturation. Effect of *Crotalaria juncea* seed extracts in male mice (Vijay kumar et al., 2004) and *Albizzia lebbeck* bark extract in male rats (Gupta et al., 2005) in their studies showed reduced protein concentration. This reduction is correlated with absence of spermatozoa in the lumen (Chinoy and Bhattachaya, 1997). Since the luminal fluid of epididymis contains a number of proteins (Brooks and Higgins, 1980). Alternation in the secretion and function of proteins impaired sperm maturation.

In the present study, protein levels in vas deferens also showed significant reduction. Similar result was reported by Vijaykumar et al., (2004), Chinoy et al., (1995 and 2005), Chinoy and Mehta (1999).

The glycogen content in the cell indicates energy storage. Chenoy and Mehta (1999) proposed that glycogen might present a source of nourishment for the spermatozoa during its development and maturation. Sertoli cells and spermatogonia often contain glycogen substrates gathered from the blood and provide source of reserve carbohydrate for seminiferous tubular cells and the glycogen level is found to be directly proportional to the steroid hormones (Pathak and Prakash, 1989). The glycogen content in the cell represents the energy storage. The sertoli cells and spermatogonia contain glycogen and provide nourishments to the seminiferous tubular cells and the glycogen content is found to be directly proportional to the steroid hormone levels (Rommerts et al, 1974). A decrease in glycogen content of the testis reduces the energy source for spermatogenic activity. The reduction in the glycogen level in testes may be due to interference in glucose metabolism. The reduced glycogen level was correlated with diminished number of post meiotic germ cells, which were supposed to be the site of glucose metabolism.
The reduced glycogen level could affect protein synthesis; because of protein synthesis in spermatogenic cells was dependent on glucose (Dixit et al., 1979). Sertoli cells and spermatogonia often contain glycogen, secrete substrates from the blood and provide source of reserve carbohydrates for seminiferous tubular cells and the glycogen level has been found to be directly proportional to the steroid hormones.

In the present study the decreased glycogen content of the testis after the administration of *Cyamposis psoralioides* extract may reduce the energy source for spermartogenic activity, which might have resulted in spermatogenic arrest. Changamma and Redanna (1985) and Sisodia et al., (2008) suggested that the decrease in glycogen content could also be due to increased glycogenolysis.

Similar results of glycogen reduction were reported after the administration of benzene and ethanol extract of *Crotalaria juncea* seeds (Vijaykumaret et al., 2004) and ethanol extract of *Amalakiyadi churna* (Seetharam et al., 2003). In male mice of extracts of *Alstonia scholaris* bark (Gupta et al., 2002) *Albizia lebbeck* bark (Gupta et al., 2005) *Barleria prionitis* roots (Gupta et al., 2000), *Semicarpus anacardium* fruits (Sharma et al., 2003), *Sarcostemma acidum* stem (Verma et al., 2002), Neem oil of *Azadirachata indica* (Parshad et al., 1997) and *Sapindus emarginatus*, *Cuminum cyinum* and *Alliu cepa* in male rats (Venkatesh et al., 2002) when compared to controls. A similar observation has been made by Bone et al., (2000) in the ornidazole treated rats. The same result has been found out by Dixit and Gupta (1982) when the compound solusocine isolated from *Sapindus trifoliatus* is administered to the dog, the glycogen in testis was decreased. The reduction in the glycogen in testis may be due to interference in glucose metabolism. The reduced glycogen level was correlated with decreased number of post-meiotic germ cells, which are thought to be the sites of glucose metabolism (Dixit and Joshi,
The reduced glycogen level could affect protein synthesis, because protein synthesis in spermatogenic cells is dependent on glucose (Dixit et al., 1979 and Gupta et al., 2000).

The present study revealed reduced glycogen contents in epididymis and vas deferens after the oral administration of *Cyamopsis psoralioides* pod extract. Similar result was found by Vijaykumar et al., (2004) who showed significant reduction in the glycogen contents of epididymis and vas deferens.

With administration of three different solvent extracts of *Crotalaria juncea* seeds resulted in reduced glycogen levels which might affect the spermatogenesis in testis and sperm maturation in epididymis and vas deferens were altered. This was probably due to impaired metabolic turn over (Chinoy et al., 1994)

Mammalian cells require cholesterol which plays an important role in acting as precursor molecule in the synthesis of steroid hormones (Gupta, 1995) and its level is related to fertility (Eik-Nes and Hall, 1962). The requirement of cholesterol for the normal activity of the testicular glands has been well established by Biswas and Deb (1965) and Johnson (1967). Group II, III and IV animals show significant decrease in the testicular cholesterol level indicating that the metabolism might have undergone alteration. This view is supported by Narayana and Chinoy (1994) after fluoride treatment to rats and also there was hypocholesterolic effect. Tributyltin (TBT) is known to disrupt the development of reproductive organs, thereby reducing fertility, down-regulate expressions of the mRNAs for cholesterol side-chain cleavage enzyme (P450scc), 17α-hydroxylase/C_{17-20} lyase (P450_{17α}), 3β-hydroxysteroid-dehydrogenase (3β-HSDH), and 17β-hydroxysteroid-dehydrogenase (17β-HSDH) were also observed after TBT exposure. Altogether, these findings demonstrate that exposure to TBT is associated with induced apoptosis of testicular germ cells and inhibition of steroidogenesis by reduction in the expression of
steroidogenic enzymes in interstitial Leydig cells. These adverse effects of TBT would cause serious defects in testicular development and function. (Kim and Kim, 2008).

The cholesterol level also decreased in epididymis after the administration of ethanolic extract of *Cyamposis psoralioides* pod. The decreased level of cholesterol in epididymis indicates the impaired functional maintenance of the epididymis same as that of administration with petroleum ether, benzene and alcohol extracts of *Momordica charantia* seeds treated either orally or intraperitoneally (Naseeem et al., 1998).

Ascorbic acid is hydrophilic and functions as a most important free-radical scavenger, trapping free radicals in the aqueous phase, thus protecting biomembranes from oxidative damage (Altuntas et al., 2002). Ascorbic acid prevents free radicals inducing DNA damage in testis (Dawson et al., 1990). It is evident from the present study, *Cyamposis psoralioides* pod extract caused decreased level of ascorbic acid in the testis of male mice, which is in consonance with that of Chatterjee et al., (1994), Chaudhary and Singh (2006) who reported hypofunctioning of testis and the degeneration of the germinal epithelium due to vitamin C deficiency. Ascorbic acid is involved in steriodogenesis of the gonads has been reported (Agarwal and Laloraya, 1977, Datta and Sanyal, 1978, Chinoy et al., 1984). It is essential for the maintenance of the structural and the functional integrity of androgen target reproductive organs (Chinoy et al., 1984).

Methyl parathion treatment to male rats induced oxidative damage in the testis and thus decreased ascorbic acid level in the testis, which was correlated with decreased sperm count and increased sperm abnormalities, indicating a close relation between them (Narayana et al., 2005). Manosodium glutamate administration to male rats showed significant decline in ascorbic acid level in testis (Nayanatara et al., 2008). Testicular ascorbic acid level also declined significantly, when swiss mice were intraperitoneally injected with lead acetate (Acharya et al., 2003). Arsenic
induced toxicity on testicular tissue of mice, which resulted in reduced ascorbic acid level (Sarkar et al., 2008).

Treatment of the fruit /seed extracts of *Sapindus emarginatus*, *Terminalia belerica*, *Cuminum cyminum* and *Allium cepa* reduced the total ascorbic acid concentration of the testis. This decrease was more significant in *Sapindus emarginatus* treated rats (Venkatesh et al., 2002).

The present investigation result in the ethanolic extract of *Cyamposis psoralioides* pods show fertility reduction by 100% in Group IV and in Group II and III by 50% when compared with control group. In the present study the fertility rate of the male depend on the dose and duration of the treatment. Similar result has been found in the treatment of *P.Guineense* to rats for 55 days which caused 20% decrease in the fertility rate (Mbongue et al., 2005). The *Dendrophthoe falcate* stem extract reduced the fertility of male rats by 87.50% at 50mg/ rat/day whereas 100% at 100 mg/rat/day dose level and 200 mg/rat/day dose levels as well as with Ionidamine (Gupta et al., 2007). 100% of fertility reduction in male albino rats (Gupta et al., 2006). 50% ethanolic extract of *Aegle marmelos* at the dose of 100 mg/Kg per day for 60 days caused 65% reduction of fertility rate and 85% in 200mg/Kg body weight per day for 60 days (Chauhan et al., 2007). Oral administration of *Kalanchoe gastonis bonnieri* natural juice to the male rats caused 50% inhibition of fertility in 150 mg/Kg body weight for 30 days and 100% fertility control in 300 mg/Kg body weight for 30 days(Beltran et al., 2003). 66.7%-100% reduction in the fertility index in rats, when injection of aqueous and steroidal extract of neem leaf (*Azadirachta indica*) in male Wistar rats (Prashad et al., 1997). 100% inhibition of fertility was found out in different plants extracts like *Juniperus phonica* (Shkukani et al., 2007), *Opuntia dillenii* (Gupta et al., 2002), *Sarcostemma acidum* (Verma et al., 2002), *Piper betle*
(Sarkar et al., 2000), Semecarpus anacardium (Sharma et al., 2003), Albizzia lebbeck (Gupta et al., 2005), Alstonia scholaris (Gupta et al., 2002) and Sapindus emarginatus (Venkatesh et al., 2002). The fertility studies reveal that the benzene extract of Ocimum sanctum leaves torats resulting in inability to fertilize the female rats probably because the male gametes are affected (Ahmed et al., 2008). Administration of Morinda lucida leaf extract resulted in a significant impairment of the treated rats fertility in terms of the number of litters born by the cohabited female rats (Raji et al., 2005). Adverse effect of the treatment of Brahmi on fertility of male mice may be due to the altered sperm parameters recorded in treated males (Singh and Singh, 2009). Etoprine was highly effective in reducing the fertility of male mice and rats (Malik et al., 1995) and also mice were infertile during the treatment of N-butyldeoxynojirimycin (NB-DNJ) (Spoel et al., 2002).

The same result was found with treatment of other different plant extracts like Echinops echinatus (Padashetty and Mishra, 2007), Dendrophthoe falcate (Gupta and Kachhawa, 2007), Ruta graveolens (Khour and El-Akwai, 2005), Strychnos potatorum (Gupta et al., 2006), Stephania hernandifolia (Ghosh, 2002), Morinda lucida (Raji et al., 2005), Azadirachata indica (Parshad et al., 1997), Quassia amara (Raji and Bolainwa, 1997) and Gossypol, a phenolic compound extracted from the cotton seed (Qian and Wang, 1984) in rats and N-butyldeoxynojirimycin (NB-DNJ) in male mice (Spoel et al., 2002), WHO reported decreased serum testosterone concentration after treatment. When Mikanaia glomerata extract was administered to male rats it showed a decrease in testosterone serum level, but not at a significant level (Sa et al., 2003). And this was similar to previously reported values (Coyotupa et al., 1973; Pujol et al., 1976 and Fahim et al., 1982).
Clinical assessment of male antifertility agents should include acceptability, safety and efficacy during and after the treatment. Such agents must have reversible antifertility effect. Our present data shows the reversible effect of the treatment. Complete recovery of fertility was observed following the withdrawal of the treatment. In the present work after the recovery period of 30 days all the animals were able to reproduce normally when compared to the control groups. Similar results have been found when oral administration of the extract of *Piper betle* Linn (Dose: 500 mg/Kg body weight/day for 30 days and 100 mg/Kg body weight/day for another 30 days) was highly effective in producing reversible functional sterility (Sarkar *et al.*, 2000). The intramuscular administration of an aqueous extract of *Carica papaya* seed caused a selective androgen deprivation resulting in infertility with complete reversibility after withdrawal of treatment (Chionoy *et al.*, 1994). Oral administration of a 50% ethanol extract of *Abrus precatorius* seeds (250 mg/Kg) in albino rats after withdrawal of the treatment caused reversible antifertility effect (Rao, 1988). Treatment with the *Curcuma longa* has caused antifertility in male mice after 56 days and withdrawal period the animals recovered to the control levels (Mishra and Singh, 2009). The withdrawal of 56 days of treatment of aqueous leaf extract of *Allamanda cathartica* to the male mice recovered when compared to the control groups (Singh and Singh, 2008). Ten weeks after the immunization of gonadotropin releasing hormone, fertilization was fully restored by 37th week in all the immunized male rats (Kumar *et al.*, 2000). The withdrawal of ethanol extract of *Carica papaya* seed in animals has shown reversible antifertility effects in male rats (Lohiya *et al.*, 1992). Treatment with the fruits of *Piper nigrum*, after the withdrawal of the period of 56 days has caused the reversibility in the animals (Mishra and Singh, 2009). Withdrawl of the treatment of seed oil extract of Iranian species of *Melia azadarach* on male rats for 3 months has showed the reversibility of the treatment (Parandin *et al.*, 2008). Withdrawl of
the treatment of Pyrimethamine for 45 days has showed reversible effect in adult mice (Kala et al., 1996). The complete recovery was found in albino rats after 120 day withdrawal of the 50% ethanolic extract of *Aegle marmelos* leaves for 60 days of treatment (Chauhan and Agarwal, 2008).

The ethanolic extract of *Cyamopsis psoralioides* was subjected to standard chemical tests as described by Kokate (1985), Harborne (1973) and Farnsworth (1977) to determine the presence (qualitatively) or absence of alkaloids, steroids, saponins, flavones and oils. The chemical tests of ethanolic extract of *Cyamopsis psoralioides* show the positive results for alkaloids, steroids, flavones, these chemical compounds help in controlling the fertility in male mice.

From the above results we can conclude that the ethanolic extract of *Cyamopsis psoralioides* pod in higher dose with the longer duration of treatment could act as a potential contraceptive agent in males.