DISCUSSION
Earlier studies on the acute toxic effects of MBC have clearly demonstrated various pathological changes in the testis of experimental animals (Nakai et al., 1992, 1998, 2002). However, there is not much information on the chronic toxicity of MBC in the testis and epididymis. Therefore, the same has been investigated in the present study. In addition, it was tested whether the reproductive toxicity of MBC was transient or permanent, and whether vitamin E could ameliorate the toxicity induced by MBC.

4.1 Body and organ weights

The absence of any significant change in the body weight of the rats studied indicate that the general metabolic conditions of the animals were within the normal range and MBC at this dose (25 mg/kg body weight) did not cause systemic toxicity. During the course of experiments, the rats did not show any variation in the food and water intake. This observation is in consonance with earlier investigations (Barlas et al., 2002; Lu et al., 2004). Further, the studies on hormonal profiles like thyroxine, growth hormone and growth factor(s) may support the contention that MBC does not affect metabolic rates in these rats.

The decreased testicular weight observed in MBC-treated rats suggests the degenerative capacity of MBC. Reduction in the weight of the testis could be due to inhibition of seminiferous tubule fluid formation and reduction in Leydig cell volume per testis, and the loss of germ cells by direct inhibition on spermatogenesis. Reduction in testicular weight is in agreement with earlier studies (Carter et al., 1987; Barlas et al., 2002). It is well known that gonadotrophins are prime regulators of testis weight (Gray et al., 1989; Goldman et al., 1989). Interestingly, in the present study, the levels of gonadotrophins were not affected significantly after MBC treatment. It appears that in situ alterations in factor(s) that are independent of gonadotrophins may be responsible for the decline in testicular weight.
It has been reported that MBC caused aspermatogenesis and depressed caudal epididymal weight (US Environmental Protection Agency, USEPA, 1999). Epididymis and accessory sex organs require a continuous androgenic stimulation for their normal growth and functions (Mathur and Chattopadhyay, 1982; Klinefelter and Hess, 1998). Hence, any change in the accessory sex organ weights may be considered to be a marker of androgen status of the animal. In the current study, the decrease in testosterone titre is in agreement with this observation. Our findings are consistent with that of earlier reports of reduction in the epididymal weight of rats exposed to MBC (Carter et al., 1987; Gray et al., 1990; Nakai et al., 1992; Correa et al., 2002).

4.2 Carbendazim levels in the serum and testis

In the present investigation, the increase in concentration of MBC in both serum and testis of MBC-treated rats indicate that the administered MBC is absorbed in the blood and accumulated in the testis. The levels of MBC absorbed are determined by factors involved in detoxification. In this regard, it is shown that the retention of MBC in rat testis is more than in mice testis, because mice possess the mechanism to prevent the toxicity (Correa et al., 2002).

Reports also revealed that greater the concentration of MBC in the testis, greater the percentage of sloughing. The severity of damage increased with the concentration of MBC metabolite at the target site, the testis, and also with time after administration (Lim and Miller, 1997). Ultrastructural studies revealed the MBC-induced testicular damages such as vacuolization, loosening of seminiferous epithelium (detachment), sloughing of Sertoli and germ cells, dispersed and hypertrophied Leydig cells and testicular atrophy.
4.3 Spermatological and Epididymidal effects

Impaired spermatogenesis is a common feature under most pesticides and fungicides toxicities. The only earlier observation with regard to spermatological assessment of the cauda epididymidis on MBC treatment was that of Nakai et al. (1992). The percentage of morphologically normal sperm in the cauda was lesser than in control, on day 4. But on day 8, many sperm were with heads separated from flagella and 10% were mishappen; sloughed germ cells were numerous and cytoplasmic debris was also evident. An increased incidence of abnormal sperm was observed on day 32, in few animals.

Further, Nakai et al. (1992) did not observe any effect of MBC on the percentage of motile sperm upto day 4 after treatment and observed marked effect on the number of motile sperm on days 8 and 16; in 3 out of 8 animals, the percentage of motile sperm still remained inhibited. In contrast, in the present investigation both motility and viability of the sperm were seriously affected. The present findings are also consistent with the earlier report of Akbarsha et al. (2001). The deleterious effect of MBC on sperm morphology and increased incidence of abnormal sperm in MBC-treated rats showed that MBC produced marked oligospermia (Gray et al., 1989). Counts, motility and viability and structural integrity of the sperm are essential prerequisites for them to be successful in fertilization. The results of the present investigation point to serious effects of MBC treatment on the cauda epididymidal sperm. This observation leads to some plausible speculations.

It is presumed that MBC has a direct access into the epididymis and probably even into the lumen. This is particularly evidenced in several sperm breaking away at the neck into head and flagellum. It is this region of the sperm, which is abundant with microtubules, belonging to the proximal centriole. The microtubule disrupting action of MBC explains the breaking away of head of the sperm from the flagellum. The effects on motility, agglutination and abnormalities of the sperm might be either caused by the
direct action of MBC or due to an indirect manifestation through altered epididymal epithelial function.

According to Burgos, (1974) the sperm leaving the testis are only structurally mature, and they are not physiologically mature. The sperm acquire motility and fertilizing ability only during their epididymal transit (Akbarsha et al., 2001). The epididymis plays an important role in the physiological maturation of sperm through the secretion of several proteins and glycoproteins. These proteins interact with the sperm in the lumen, which involve addition of new proteins to the sperm surface, deletion of some of the existing proteins, and modification of some other proteins (Cooper, 1992). The epididymal epithelium is also concerned with a tremendous modification of the fluid and solute concentrations in the lumen, and such modified concentrations can affect the sperm. The function of epididymal epithelium is suggested to be altered with the modification of the fluid and solute concentrations in the lumen (Ilio and Hess, 1994).

Therefore, a small dose of MBC (25 mg/kg body weight) given daily for 48 days brings about dramatic changes in the epididymis and impaired epididymal maturation of sperm. Thus, MBC appears to have direct action on the epididymis and causes extensive damage to the sperm.

4.4 Histological studies of testis

Benzimidazole compounds such as benomyl and its metabolite MBC are two of the best characterized Sertoli cell toxicants known to cause premature sloughing of germ cells along with cleaved cytoplasmic processes of Sertoli cells (Nakai and Hess, 1994), necrosis of meiotic spermatocytes (Parvinen and Kormano, 1974). Nakai et al. (1992) were the first to suggest that this sloughing of affected portions of Sertoli cells were due to its fungicidal property. The fungicidal property enables MBC to bind to microtubules through tubulin leading to polymerization and inhibition of mitosis (Davidse and Flach, 1977; Burland and Gull, 1984). In the mammalian system,
this mechanism has been proposed to explain long-term atrophy observed in the testis of MBC-treated rats (Carter and Laskey, 1982; Carter et al., 1987; Gray et al., 1990).

The second major mechanism of action of MBC in the sloughing of seminiferous epithelium resides in the disturbance caused to the microtubules of the Sertoli cells. They have branching cytoplasmic processes, and the differentiating spermatogenic cellular elements associated with the Sertoli cells. These Sertoli cells are always associated with proliferating germ cells (Bardin et al., 1994). Sertoli cellular elements are extending from the basement membrane to the lumen of the seminiferous tubules. The shape of Sertoli cell is roughly tall and columnar, and within its basic organization the cell undergoes cyclical change in response to or corresponding to the differentiation of spermatogenic cellular elements (Wong and Russell, 1983; Russell, 1993; Nakai et al., 1995). Nakai et al. (1995) observed that Sertoli cells in tubules with MBC-induced sloughing were somewhat conical in shape. This is confirmed in the present investigation also. Nakai et al. (1995) suggested that when Sertoli cells are sloughing there is general displacement of cytoplasm towards the base of the cellular axis. The increase in the number of round-shaped and perpendicular positioned nuclei resting along the basement membrane is the probable result of this displacement, and there is a strong possibility that it is due to loss of microtubules in the Sertoli cells (Nakai et al., 1995).

The present results relating to fibrosis of the seminiferous tubules are interesting. The well-established mechanism of action of MBC in the spermatogenic compartment is the sloughing of the Sertoli cell, ultimately resulting in complete atrophy of the seminiferous tubules (Nakai et al., 1992). If this is the only and indiscriminate mechanism, the kind of fibrosis observed will not be possible. Kadalmani et al. (2002) noticed that in the fibrotic seminiferous tubules, the entire Sertoli cells were not only intact but their processes were more ramified and filled the entire seminiferous tubule leaving
little lumen when rats were exposed to MBC. Perhaps, the results signify that the ultimate reflection in the seminiferous tubule is based on the Stage at first exposure of the seminiferous tubule to MBC. First exposure at certain stages resulted in sloughing of apical portion of the Sertoli cell at different levels along the height in a stage dependent manner, whereas at some other stages it may result in retention of Sertoli cell, causing premature exfoliation of the germ cell resulting in Sertoli cell fibrosis (Kadalmani et al., 2002). The present study also confirms the similar trend.

Carter et al. (1987) observed that the seminiferous tubules of rats when exposed to MBC showed “Sertoli cell-only syndrome” in which all germ cells were missing in the seminiferous tubules. They suggested that once a tubular basement membrane has thickened, that portion of the tubule may no longer be available for normal spermatogenesis and also may account for the atrophic tubules. Although Sertoli cell only syndrome-like situation prevailed along with seminiferous tubular atrophy due to MBC treatment, abnormal spermatogenesis was also seen in the present investigation.

**Inter-Sertoli cell and Sertoli cell-germ cell associations** are critical aspects of spermatogenesis as they constitute the blood-testis barrier and tubulobular complexes. Both are formed due to the ectoplasmic specializations (ES). In the development of ES, the microtubules, vimentine filaments, actin microfilaments and the endoplasmic reticulum of the Sertoli cell cytoplasm participate. This forms a complex and plays critical roles in holding the germ cells between Sertoli cells and their vertical displacement consequent upon division and differentiation of the germ cells (de Kretser and Kerr, 1994). Nakai and his associates (1995) have shown that MBC causes disruption of microtubule of the cytoskeleton of the body of the Sertoli cells. This result in the apical portions of the Sertoli cells breaking away and such fragments carry with them the associated germ cells, which reach the lumen of the seminiferous tubules and arrive at the ductus epididymidis (Nakai and Hess, 1994; Nakai et al., 1995; Kadalmani et al., 2002). Therefore, the Sertoli cells fragments
noticed in the lumen of the ductus epididymidis, in this study, might have been produced due to the disruption of the cytoskeletal framework of the Sertoli cells and thus causing depletion of the germ cells in the seminiferous epithelium. Thus, it could be concluded that the route of entry of MBC into the seminiferous tubules is, in all probability, through the Sertoli cell, since there is also an impact of MBC on the germinal cells of the basal compartment.

The present study has clearly established that one of the cellular mechanisms of action of MBC in the testis is generation of multinucleate giant cells. Russell et al. (1987) demonstrated for the first time that loss of integrity of the intercellular bridges between male germ cell clones as the mechanism for production of multinucleate giant cells or symplasts. Spermatids being haploid cells, the cytoplasmic bridges are meant for equal distribution of gene products among the cells of the clone and thus provide for genetic equality between the haploid cells (Zheng et al., 2001). The present study provides exquisite and unambiguous ultrastructural evidence of opening of cytoplasmic bridges towards the formation of multinucleate giant spermatids. Therefore, it is suggested that MBC toxicity directly or indirectly targets the actin microfilaments of the cytoplasmic bridges, which connect clones of spermatids.

As pachytene is the longest phase in meiosis, it is vulnerable for the actions of MBC. MBC induces fragmentation of chromatin in pachytene spermatocytes and nuclear pycnosis. The histological and ultrastructural approaches in the present study have clearly revealed that necrosis is the cause for germ cell death due to MBC treatment. Necrosis is the case of cytoplasmic swelling, karyolysis and rupture of the cells releasing the content onto the immediate surrounding. Thus, the disruption caused to the chromosomes/chromatin/DNA as well as the microtubules of the spindle apparatus of male germ cells ultimately causes cell death. The dead cells either rupture and release the content (oncotic necrosis) or undergo compaction (apoptotic necrosis) and are prematurely released from the Sertoli cell.
The adverse effects of MBC on Leydig cellular ultrastructural changes seem to be a new finding. The functional impairment of Leydig cells is evident from the decline in the activities of steroidogenic enzymes and testosterone production. A consequential decrease in serum testosterone is in agreement with an earlier study (Barlas et al., 2002). The original contribution in the present study in this regard relates to the pathological changes in the Leydig cell. In aflatoxin-treated rats, the androgenic machinery consisting of smooth endoplasmic reticulum and mitochondria (Eddy and O'Brien, 1994) are thoroughly affected, understandably hampering androgen synthesis. The impairment of androgen synthesis is further substantiated in two opposing situations of accumulation of lipid and depletion of lipid. Cholesterol and its esters are the raw materials for synthesis of androgens, and their depletion/accumulation reflects on impairment of androgen synthesis (Eddy and O'Brien, 1994). Nakai and Hess (1994), found mitochondrial and endoplasmic reticulum swelling in the rat testis during MBC treatment. The present study clearly indicates that the mitochondria of Leydig cells are oedematous and undergo severe pathological changes. In addition to these changes, steroidogenic pathway was also affected in the MBC-treated rats.

The pathological changes observed in Leydig cells of MBC-treated rats can also be interpreted in the light of the imminent impact of MBC on the architecture of the Leydig cells. These changes include hypertrophy, distortion of shape of nucleus and elongated, increased vacuolation and osmotic swelling of mitochondria and endoplasmic reticulum. The effect of MBC on the microtubules of the spindle fibre and the actin microfilaments of the cytoplasmic bridges has already been discussed.

4.5 Pituitary-Testicular Axis

Pituitary and hypothalamus are considered as target organs for reproductive toxicants/endocrine disruptors (see Cooper and Goldman, 1998;
see Tucker, 1999). Any endocrine alterations that occur at the levels of hypothalamus and pituitary may contribute to the reproductive failure.

The unaltered levels of serum LH, FSH and prolactin in MBC-exposed rats suggest that pituitary-hypothalamic function was not altered significantly by MBC and that the observed testicular effects were likely due to the direct action of MBC on testis. Earlier studies have implied that the adverse effect of MBC on fertility in male rats is due to its effects directly on the testes (Parvinen and Kormano, 1974; Barnes et al., 1983; Carter et al., 1984; Linder et al., 1988). In contrast to the present study, MBC has been reported to increase serum gonadotrophins in an earlier study (Goldman et al., 1989). There was a daily treatment of MBC for 85 days in the study of Goldman et al. (1989), while in the present study daily treatment of MBC was for 48 days only. Further, they have used 21 days old Long-Evans hooded rats. However, in the present study 90 days old Wistar strain adult rats were used. Moreover, the dose given in the present study was 25 mg/kg body weight/day only while Goldman et al. (1989) had administered higher doses viz., 50, 100, 200 or 400 mg/kg body weight/day. The probable reason for the observed inconsistency between the present study and that of Goldman et al. (1989) may be the dose, duration of MBC treatment and species specificity. In the present investigation, the lack of change in serum LH, FSH and prolactin may suggest that the given dose of MBC (25 mg/kg body weight/day) might not be sufficient to alter the secretion of gonadotrophins and prolactin.

In the absence of any obvious alteration in gonadotrophins, the remarkable decrease in the activities of steroidogenic enzymes studied and androgen production clearly demonstrate the direct adverse effects of MBC on Leydig cell structure and function. Testosterone is the principal androgen secreted by the Leydig cells. Testosterone promotes the production of functional sperm, maintains the secretory glands of the male reproductive tract and stimulates growth. The observed decrease in serum testosterone in MBC-treated rats may be a cumulative effect of decreased Leydig cell number,
testosterone synthesis and secretion. Probably, the reduction in serum testosterone levels could also be due to the diminished responsiveness of Leydig cells to LH and/or the direct inhibition of testicular steroidogenesis. It is well established that LH is the prime regulator of testosterone production by the Leydig cells. In the present study, testosterone levels were decreased in the serum, despite no significant change in the serum LH titre. This may be due to the defective signal transduction of LH that again depends at various levels like LH receptor, second messenger or other down stream molecules in LH signaling pathways. However, it needs further study to arrive at a definite conclusion.

It is well known that 3β-HSD, which is the key enzyme that catalyses the conversion of pregnenolone to progesterone and 17β-HSD, which is necessary for the formation of testosterone from androstenedione (Mendis-Handagama, 2000). Leydig cellular 3β-HSD and 17β-HSD activities were lowered in the MBC-treated rats. The decreased steroidogenic potency in the MBC-treated rats was also observed in serum testosterone titre. The catalytic activity of glucose-6-phosphate dehydrogenase (G6PDH) is associated with the generation of NADPH, a co-factor essential for steroidogenesis. The reduced steroidogenic enzyme activity in MBC-treated rats correlates with the reduction in the activity of G6PDH observed in the present study. The decreased activities of steroidogenic enzymes studied (3β-HSD and 17β-HSD) in the MBC-treated rats may also be due to the increased production of LPO and ROS in the Leydig cells. Though earlier investigators did not determine the activities of steroidogenic enzymes, this may be the first observation on the activities of steroidogenic enzymes due to MBC treatment.

The reduction in serum estradiol noticed in MBC-treated rats may be due to impaired synthesis or enhanced metabolism. It is evident that the synthesis of estradiol from testosterone depends on the activity of aromatase, an enzyme responsible for maintaining the homeostatic balance between
androgens and estrogens in both sexes. The reduction in the serum estradiol in the present study may be due to a decrease in the activity of the enzyme aromatase, consequent to the diminution of the substrate, testosterone. Various pesticides like hepaticlor, cypermethrin, ketoconazole (Gray et al., 1999), and imidazole fungicides (Vinggard et al., 2002) have shown to suppress aromatase activity. Probably MBC could have acted as an aromatase inhibitor. Thus, MBC treatment has not only suppressed the production of testosterone but also interfered in the formation of estradiol.

4.6 Leydig cellular Pro-oxidant and Antioxidant System

The present study revealed that exposure to MBC has adverse effects on the Leydig cellular enzymatic and non-enzymatic antioxidant defense systems. Environmental pollutants may induce oxidative stress leading to the generation of free radicals and alterations in antioxidants (El Sharkawy et al., 1994).

During normal steroidogenesis, LPO and ROS are produced by electron leakage outside the electron transfer chains and these oxygen radicals can initiate lipid peroxidation, to inactivate P450 enzymes (Hanukoglu et al., 1993). Several lines of evidence indicate that interactions between the testicular antioxidant and steroidogenic enzyme systems are complex and physiologically relevant. Girotti (1985) suggested that the free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation. Recently, Zini and Schlegel (2003) reported that androgen deprivation induced lipid peroxidation in rat testis. The decrease in serum testosterone and increased LPO observed in the present study are in agreement with this earlier report.

The enzymatic and non-enzymatic antioxidants are the natural defense system against free radical mediated tissue damage in several organs including testis. Oxygen free radical (OFRs) enzymatic scavengers like SOD, CAT, GPx, GST, GR, γ-GT and G6PDH may protect the system from deleterious effect of OFRs (Banerjee et al., 1999). The decrease in activities of enzymatic
antioxidants in MBC-exposed rat Leydig cells might have increased LPO and ROS production. SOD is responsible for the dismutation of superoxide radical to hydrogen peroxide, which is neutralized by the combined action of CAT and GPx in all vertebrates (Mates, 2000). These enzymes act in coordination and the cells may be pushed to oxidative stress state if any change occurs in the levels of enzymes. The decreased level of SOD activity in MBC-exposed rat Leydig cells, suggests an increased superoxide radical production.

It has been reported that the enzyme catalase is found in the peroxisomes of Leydig cells (Mendis-Handagama, 2000). In addition, Leydig cell peroxisomes participate in the intracellular cholesterol trafficking and delivery into mitochondria during LH-stimulated steroidogenesis in adult rat (Mendis-Handagama, 2000). In the present study, reduced catalase activity in Leydig cells of MBC-treated rats suggests that these Leydig cells have reduced amount/volumes of peroxisomes that may affect the process of steroidogenesis. The decreased activity of GPx in the Leydig cells reveals that MBC might have inhibited the enzyme directly by impairing the functional groups, or indirectly by rendering the supply of reduced glutathione (GSH) and NADPH insufficient needed for its action.

Further, GST provides protection to the tissues by catalyzing the conjugation of a variety of electrophilic xenobiotics to GSH (Chasseaud, 1979). Thus, the decreased activity of GST indicates the direct interaction of MBC with this enzyme. GR and G6PDH enzymes play an important role in maintaining glutathione redox state. The decrease in the activity of G6PDH in the present study suggests the reduced supply of NADPH for catalytic activity of GR. Consequently, there was fall in the GR activity. γ-GT couples the gamma-glutamyl moiety to a suitable aminoacid acceptor for transport into the cell and make it available for intracellular GSH synthesis, since most cells are unable to take up the intact form (Markey et al., 1998). The observed decrease in Leydig cellular γ-GT activity in MBC-treated rats indicates the suppressed synthesis of GSH. It has been reported that the increased concentration in
malondialdehyde, an end product of LPO was associated with iron-induced rat testis injury (Merker et al., 1996). The reactions of hydrogen peroxide with transition metals like Fenton reaction results in the generation of extremely reactive hydroxyl radicals (Cao et al., 2004).

In mammalian cells, mitochondria are considered to be the major source of free radical formation as a by-product of oxidative phosphorylation (Raha and Robinson, 2001). Free radicals are also generated during steroidogenesis (Hornsby, 1989; Peltola et al., 1996). Thus the rise in free radicals in MBC-exposed rat Leydig cells might render mitochondrial steroidogenic machinery and StAR proteins more susceptible to lipid peroxidation and oxidative damage leading to their functional inactivation.

GSH quenches toxic hydroperoxides catalyzed by glutathione peroxidases and some peroxiredoxins, and conjugation reactions catalyzed by glutathione-S-transferases (Chasseaud, 1979). In the present study, the decreased level in GSH induced by MBC could seriously impair the optimal functioning of these various catalytic activities (Mates, 2000). Vitamin E is the only most effective chain-breaking, lipid-soluble antioxidant weapon that inhibits lipid peroxidation induced by ROS (Dieber-Rotheneder et al., 1991; Palamanda and Kehrer, 1993; John et al., 2001; Gupta et al., 2004). The reduced levels of vitamin E in the present study indicates the subnormal scavenging of lipid peroxidation and also suggest the excessive utilization of this antioxidant for quenching enormous free radicals produced in MBC-exposed rats.

Ascorbic acid is a water-soluble antioxidant, which exists, in higher concentration in many mammalian tissues including steroidogenic tissues like the adrenal gland (Das et al., 1993). It scavenges many different types of free radicals including superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen and reactive nitrogen species and is also capable of regenerating α-tocopherol from the tocopheroxy radicals that are generated during the α-tocopherol mediated inhibition of lipid peroxidation (Dieber-Rotheneder
et al., 1991). The reduction in vitamin C in MBC-exposed rats may be due to the decrease in the recycling of ascorbic acid (i.e.) due to a decrease in the conversion of dehydroascorbic acid to ascorbic acid, since vitamin C is converted to dehydroascorbic acid during its antioxidant function (Rumpsey and Levine, 1998). The reduction in vitamin C in rats exposed to MBC could have contributed to the damages to the reproductive functions in MBC-treated rats.

Vitamin A is a fat-soluble antioxidant, which is essential for growth, maintenance of visual function, reproduction and differentiation of epithelial tissue. Carotenoids are known to quench oxidant species such as singlet molecular oxygen; the mechanism seems to be a physical quenching reaction that does not affect chemically the structure of the pigment (Ray and Husain, 2002). It has been reported that vitamin A deficient rats exhibited lowered serum testosterone while LH remain unaltered, and supplementation of retionionic acid to this vitamin A deficient rats restored normal testosterone concentration (Appling and Chytil, 1981). Rats fed with excess vitamin A showed decreased level of LPO and increased SOD and CAT activities suggesting that excess vitamin A supplementation in diet functions synergistically with high protein diet to reduce LPO and to increase antioxidant enzymes (Karar et al., 2002). The decrease in Leydig cellular concentration of vitamin A in the present study underscores the adverse effects of MBC on Leydig cellular functions.

4.7 Leydig cellular Lipid profiles

The present results indicate that MBC-induced changes in lipid profiles may lead to the alteration in Leydig cellular structure and function. MBC possess a high permeability across the lipid bilayer membrane, which facilitates the fungicide diffusing into the extravascular compartment by either entering cells or being bound to some extravascular structures (Jia et al., 2002). Lipids, apart from serving as the main source of energy also have an important role as
the precursors of steroidogenesis in Leydig cells. The decrease in total lipids can be attributed to the reduction in cholesterol, glyceride glycerols and phospholipids. This is due to the fact that phospholipids and neutral lipids are the major lipid classes in the Leydig cells (Johnson, 1970; Gunasekar et al., 1991). Further, the decreased levels of total lipid, total cholesterol, total glyceride glycerols and total phospholipid in the present study may be due to the lipid peroxidative damage caused by MBC.

Since the cholesterol fractions of lipids form the precursor in steroidogenesis, the same has been considered for estimation and interpretation. Cholesterol required by the Leydig cells for the testosterone synthesis is derived from high-density lipoprotein (HDL) (Anderson and Dietschy, 1978) and also by de novo synthesis in Leydig cells (Charraeau et al., 1981; Pignataro et al., 1983). A small portion of cholesterol is esterified by acyl CoA:Cholesterol:acyl transferase stored as lipid droplets and serve as a reservoir of cholesterol (Mori and Christensen, 1980) and the lipid droplets are depleted during normal spermatogenesis in the testis. Majority (90-95%) of cholesterol occurs free whereas only 5-10% is esterified (Oshima and Carpenter, 1968). Cholesterol being a precursor of steroid hormones, a marked decrease in its content in Leydig cells on MBC treatment implies inhibition of steroidogenesis in Leydig cells. Generally, the active steroidogenesis will be associated with reduction in esterified cholesterol with more free cholesterol for utilization (Perlman, 1950). Interestingly, in the present investigation there is a significant decrease in testosterone with reduced concentrations of both free cholesterol and esterified cholesterol.

Luteinizing hormone (LH) accelerates steroidogenesis by stimulating some steps beyond the cholesterol synthesis (Bex and Bartke, 1977; Zipf et al., 1978; Purvis et al., 1979; Aruldhas et al., 1986). In the present study, both forms of cholesterol are at low level without any significant alteration in serum LH level. Therefore, decrease in testosterone may be the outcome of the direct action of MBC on the Leydig cells. The decreased concentrations in
total, free and esterified cholesterol suggest the lowered cholesterol biosynthesis. The decreased level of cholesterol is reflected in decreased steroidogenic enzymes activity and testosterone level, since cholesterol is a precursor in testosterone biosynthesis and its level is closely related to the fertility and the sperm output (Nair et al., 1987).

In conclusion, a marked decrease in the lipid classes observed in the present investigation on MBC treatment may be associated with the impairment of testicular function.

4.8 Co-administration of vitamin E with MBC

The reproductive toxicity induced by MBC primarily involves the induction of oxidative stress. In view of this, it is decided to study the ameliorative effect of vitamin E. In order to know whether the selected dose of vitamin E has any adverse effect on the normal structure and function of testis, a group of rats were given vitamin E alone. Interestingly, in none of the parameters studied, vitamin E exerted any adverse effects. These findings indicate that vitamin E does not intervene with normal testicular structure and function. However, it appears to be active and ameliorative in preventing the toxic effects of MBC, when it was given along with MBC.

Co-administration of vitamin E with MBC resulted in complete prevention of the deleterious effect of MBC in testis, epididymis, seminal vesicles and ventral prostate weights. It is clearly evident in the rats treated with vitamin E and MBC, which showed normal seminiferous tubules possessing intact seminiferous epithelium with spacious lumen and intact cell types viz., Sertoli, germ and Leydig cells. The absence of any significant change in the levels of serum testosterone, estradiol, and activities of steroidogenic enzymes in the rats co-administered with vitamin E and MBC indicates the protective effect of vitamin E.
Vitamin E is the only most effective chain-breaking, lipid soluble antioxidant weapon against lipid peroxidation (Dieber-Rotheneder et al., 1991). Vitamin E has been successfully used to provide protection against oxidative stress in different tissues including testis (John et al., 2001; Gupta et al., 2004). Co-administration of vitamin E with MBC protects the testicular Leydig cells against lipid peroxidation induced by MBC by increasing enzymatic and non-enzymatic antioxidants and decreasing LPO and ROS generation. Vitamin E is known to protect cells from diverse actions of free oxygen radicals by donating its hydrogen atom.

The observed protective effects of vitamin E from MBC induced oxidative stress are consistent with earlier investigations that used pesticides such as tolclophos-methyl (Orth et al., 1993), dimethoate and malathion (John et al., 2001) in rats. It is also reported that α-tocopherol suppressed the production of reactive oxidants and partly improved sperm quality (Aitken and Clarkson, 1987). Beside its antioxidant function, vitamin E is known to stabilize polysaturated fatty acids (PUFA) with the cell membrane, thus assuring the normal fluidity of the lipid bilayer. Thus co-administered vitamin E protects the testis by upregulating the antioxidant system.

4.9 Withdrawal of MBC-treatment

In the present investigation, restoration of normal testicular function in rats was achieved after withdrawal of MBC treatment. The findings indicate that adverse effects of MBC treated for 48 days can be reversed by a similar duration of withdrawal of treatment. It is interesting to note that almost all parameters that were affected by MBC treatment were restored to normal upon withdrawal of treatment. This unique observation emphasizes that MBC could have turned on one particular mechanism to execute all the adverse effects observed. The mechanism appears to be MBC induced oxidative stress. The elevated LPO and the decline in the levels of antioxidants are consistent with this notion.
Further, the findings imply that the adverse effects of MBC are transient and reversible. A similar reversible effect of benomyl on epididymal sperm count and motility were reported (Toppari et al., 1996; Barnes et al., 1983). These authors reported complete reversal of benomyl (1.0, 6.3 or 203 ppm, 70 days feeding course) induced suppression of epididymal sperm count, motility and testicular atrophy, when rats treated with benomyl were kept for a further 70 days period under benomyl-exposure free condition.

In conclusion, chronic low dose treatment of MBC is capable of inducing reproductive toxicity through increased oxidative stress, but is transient and reversible upon withdrawal of treatment. Further vitamin E, an antioxidant by itself quenches the oxidative stress and protect the male reproductive organs from the onslaught of oxidative stress.