Recent Research in Science and Technology 2010, 2(8): 07-17
ISSN: 2076-5061
www.recent-science.com

PHARMACOLOGY

EFFECT OF MANCOZEB ON THYROID, TESTIS, ACCESSORY REPRODUCTIVE ORGANS AND BIOCHEMICAL CONSTITUENTS IN ALBINO MICE

Raghavendra L. Ksheerasagar[1] and Basappa. B. Kaliwal[2]*

[1]*Department of Biotechnology, C.M.R.I.T, Bangalore, Karnataka, India*
[2]*P.G. Department of Studies in Biotechnology and Microbiology, Karnatak University, Dharwad – 580 003, India*

Abstract

Mancozeb, a fungicide of ethylenebisdithiocarbamate group was orally administered at doses of 200, 400, 600 and 800 mg/kg/ day to male swiss albino mice for 30 days. Daily body weights of the mice were recorded and the mice were sacrificed on the 31st day. Testes weight decreased significantly in all the mancozeb treated mice except 200 mg/kg/ day treated mice. Mice treated with 600 and 800 mg/kg/ day showed significant decrease in the number and diameter of spermatogenic cells and Leydig cells. However, treatment with 600 mg/kg/ day revealed no significant change in the diameter of spermatogonia and primary spermatocytes. Histological studies of the testis of the mice treated with high doses of mancozeb revealed spermatogenesis inhibition reflected by significant decrease in number of spermatogenic cells and sperms. The thyroid effect of mancozeb is revealed by the number of small follicles decreased where as the number of medium and large follicles increased significantly with hypertrophy and hyperplasia of follicular cells with loss of colloid in mice treated with high doses of mancozeb when compared with corresponding parameters of control. Mice treated with 800 mg/kg/ day mancozeb showed significant decrease in the weight of prostate and Cowper's glands. Mice treated with 600 and 800 mg/kg/ day mancozeb showed significant decrease in the kidney, spleen and liver weight, where as thyroid weight increased significantly. However, thymus weight increased significantly in the mice treated with 600 and 800mg/kg/ day mancozeb. Mice treated with 200 and 400 mg/kg/ day mancozeb caused no significant change in the biochemical constituents in testes, liver and kidney. However treatment with 600 mg/kg/ day mancozeb caused significant decrease in the level of protein in liver and glycogen in the liver and kidney and a significant increase in the level of total lipids in testis. Treatment with 800 mg/kg/ day mancozeb caused significant decrease in the levels of protein and glycogen and significant increase in the level of total lipids in the testis and liver and a significant decrease in the protein, glycogen and total lipids in the kidney. These observed effects of mancozeb on testis, thyroid and biochemical constituents may be due to hormonal imbalance in any of the stages in the hypothalamo-hypophysial-testicular axis or hypothalamo – hypophysial –thyroid axis.

Keywords: Mancozeb, Testis, Thyroid, Mice, Toxicity

Introduction

Mancozeb (Manganese ethylene bis dithiocarbamate polymeric complex with zinc salt) a carbamate from the class of fungicides used against a variety of foliar fungal diseases and for seed treatment. Studies on the reproductive effects of carbamate pesticides are of immense importance in the field of toxicology. Carbamates are chosen on the basis of their properties of biodegradability with low mammalian toxicity Mancozeb shows its biological effects through it's metabolites like ethylene thiourea (ETU) and carbon disulphide (CS2)/1/. Carbon disulphide causes significant decreases in serum testosterene, marked degenerative changes in testicular tissue, affects spermatogenesis and also causes epididymal alterations/2/. Trivedi *et al*/3/. have reported mancozeb at doses 500, 1000, and 1500 mg/kg/ day to the rats for 90 days caused hypertrophy and hyperplasia of thyroid follicular cells. The ETU one of the major metabolite of mancozeb relatively accumulates in thyroid irrespective of way of exposure/4/. Mancozeb rapidly degrades to ethylene thiourea (ETU) in presence of water and oxygen .Mancozeb rapidly absorbed into the body from gastrointestinal tract, distributed to various target organs and takes 96 hours to be excreated completely. The ETU comprises almost 24% of bio-available dose in urine and bile/5/. Mouse metabolizes ETU preferentially via the flavin dependent mono-oxygenase (FMO) system. The FMO mediates binding of ETU metabolites to mouse liver proteins may contribute to the hepatotoxicity/6/.

It has been revealed that the administration of N-methyl dithiocarbamate inhibits the secretion of lutenizing hormone thus affecting ovulation in rat/7/ .It has been also reported that the carbamate fungicide carbendazim has rapid direct effects on meiotic spermatocytes and latent effects on spermatids leading to morphological abnormalities and failure of spermatogenesis in rats/8/. Carbendazim induces chromosome abberation in spermatids with high indices of aneuploidy/9/. Kitagawa et al., /10/ have reported that oral administration of 3 mg carbaryl per week for 365 days to rats reduced the number of

* Corresponding Author, *Email*: b_kaliwal@yahoo.com, raghu.lksagar@gmail.com

spermatogonia and spermatozoa. Several studies indicated that hypothyroidism is associated with diminished libido and impotence in men/11, 12, 13/. Beamer et al., /14/ have reported the infertility of hypothyroid male mice could be reversed by food supplemented with desiccated thyroid powder. Similarly Jiyang et al., /15/ have reported thyroxine treatment to the infertile *rdw* rats caused recovery in testicular and epididymal weights with increased serum thyroxine(T4) levels. The sexual behaviour testicular and epididymal function brought to normal. Complete reversion was determined both invitro and invivo for fertilization without loss of sperm viability. There for the present investigation was aimed to study the effect in mice with 30 days exposure of graded doses of the carbamate fungicide mancozeb on thyroid weight, thyroid follicular dynamics, testicular weight, testicular diameter, percent defective seminiferous tubules with hypospermatogenic condition, weight of accessory reproductive organs, vital organs and biochemical constituents of testis, liver and kidney in albino mice.

Experimental

Chemical

Mancozeb (commercial grade 75% wettable powder) was made available from Indofil chemicals company, Mumbai and dissolved in olive oil for oral administration. Doses were given according to their daily body weight.

Animals

Male Swiss albino mice (80-90 day old) weighing 25-30 gms were used for the experiment. The mice were maintained in separate cages under controlled conditions of temperature ($26^0 \pm 1^0$ C), and light (12h light: 12 h dark cycle). Animals were given synthetic pellet diet 'Gold mohar" (Hindustan Lever Company, Mumbai) and water provided *adlibitum* throughout the study. The animals were divided into five groups (n=10 per group).The doses given were below the acute LD50 level of intoxicated and up to 1/10th value of LD50. Mancozeb in doses of 200, 400, 600 and 800 mg/kg/ day was administered orally for 30 days to respective groups. Olive oil treated mice served as controls.

Assessment procedures

Body and organs weight studies

All the animals were killed on the 31st day after the last dose treatment. The percent change in body weight was calculated on the basis of the initial body weight taken on first day after the oral administration and final body weight taken on last day of treatment. The testes, epididymides, vasa deferentia, seminal vesicles, prostate glands, Cowper' glands, coagulatory glands were dissected out. The adherent fatty tissues and blood vessels were removed blotted free of mucous and weighed to the nearest milligram. Kidney, adrenals, liver, spleen, thymus, thyroid were also dissected out and weighed. To ensure normalization of data for statistical analysis, organs weights were expressed per 100 g body weight.

Histological studies

The testis and thyroid were fixed in Bouins fluid embedded in paraffin and sectioned at 5 m thickness and stained in haematoxylin-eosin. Sections were examined under the light microscope and general histological appearance was assessed. From each testis 10 sections were randomly selected for histologic and histometric observations were made with a calibrated ocular micrometer. In each section ten seminiferous tubules exhibiting round shape between II to VIII stages were selected in accordance with the criteria of Leblond and Clermont /16/. The seminiferous tubules were examined for counting the different spermatogenic cells and Leydig cells lying around them. The diameter of spermatogenic cells and Leydig cells were determined after 1000 observations of particular cell types per testis from each animal of control and treated groups. Spermatogonia, primary spermatocytes, secondary spermatocytes, round spermatids and Leydig cells were identified as per findings of earlier investigators /17,18,19,20/ as reviewed by deKrester and Kerr /21/.The data were expressed as number or diameter of spermatogenic and Leydig cells per seminiferous tubule. Serial sections of thyroid gland were observed, number of thyroid follicles of different size were counted as small of below 30µm, medium of 30 - 90µm and large above 90µm diameter.

Biochemical Studies

Freshly removed testis, liver and kidney tissues were weighed to required milligram for biochemical analysis such as protein, glycogen and total lipids. The net weights of the tissues were estimated gravimetrically. Protein estimation was performed as per the method described by Lowry et al., /22/, glycogen by Scieffer et al., /23/ and total lipids by Folch et al., /24/.

Statistical analysis

Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett's test ($P<0.05$).

Results

Testes and accessory sex organs weight studies

Oral administration of the mancozeb caused significant decrease in the weight of testes with increasing doses of mancozeb except 200 mg/kg/ day.

Treatment with 400 mg/kg/ day mancozeb caused significant decrease in prostate gland weight. Simultaneously treatment with600 and 800 mg/kg/ day mancozeb caused significant decrease in the weight of prostate and Cowper's glands (Table 3). There was no significant change in the weight of the epididymides, vasa deferentia, seminal vesicles, coagulatory glands in all the mancozeb treated mice when compared with controls (data not shown).

Histologic studies

Mice treated with 600 and 800 mg/kg/ day showed significant decrease in the number and diameter of spermatogenic cells and Leydig cells. However, treatment with 600 mg/kg/ day revealed no significant change in the diameter of spermatogonia and primary spermatocytes (Table.1). Histologic observation of the testes showed normal spermatogenesis with spermatogenic cells at different stages of development in control mice (Fig. 1). Histologic examination of the testes with low dose of 200 mg/kg/ day mancozeb showed normal spermatogenesis with spermatogenic cells and interstitial tissue consists of Leydig cells (Fig. 2) Histologic observations of the testis with increasing doses of 400, 600 and 800 mg/kg/ day mancozeb revealed spermatogenesis inhibition reflected by significant decrease in the number of spermatogonia, spermatocytes, spermatids and formation of many giant cells with less sperms in the lumen of seminiferous tubules (Fig. 3 to 5).

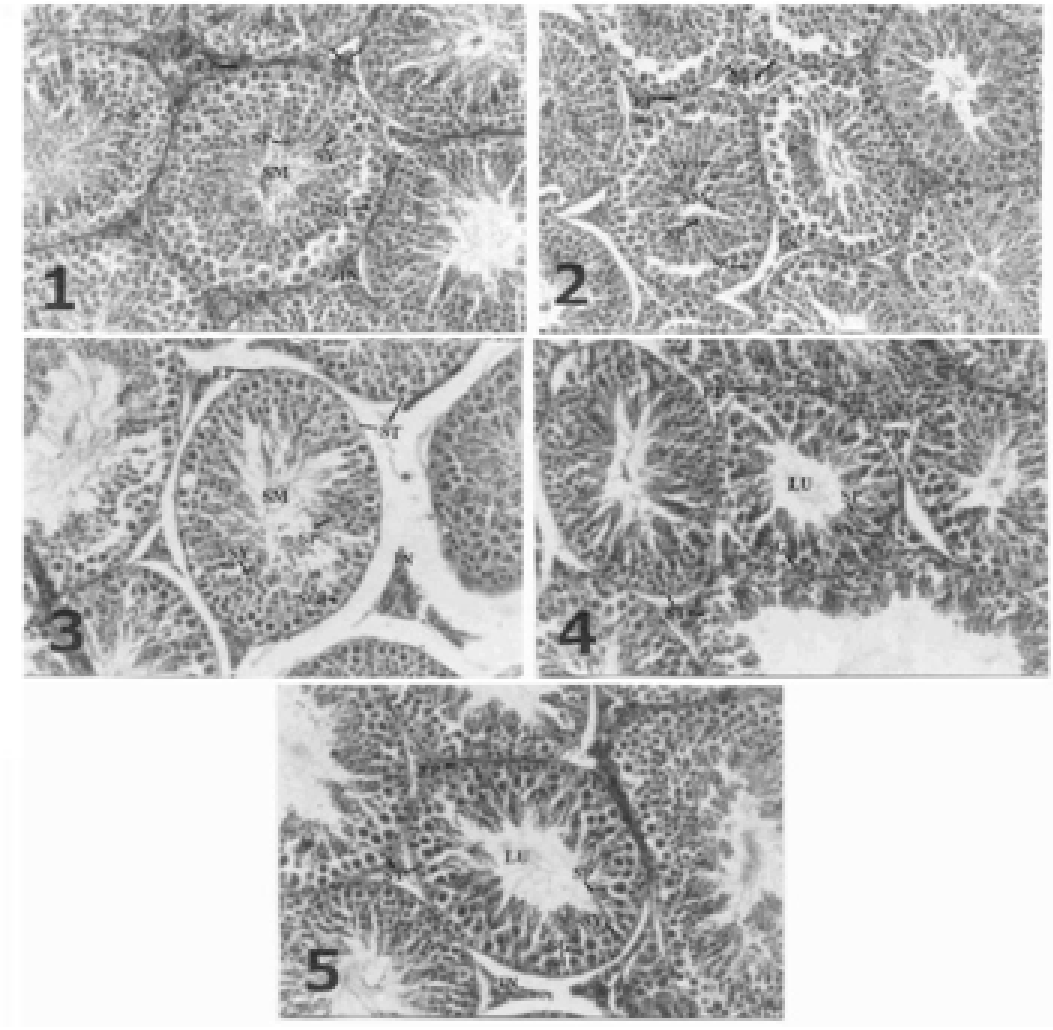

Fig 1: Testis of the vehicle-treated (control) mouse showing different stages of spermatogenesis. HE X 200. Fig 2: Testis of the mouse treated with mancozeb (200mg/kg/ day) showing normal spermatogenesis. HE X 200. Fig 3: Testis of the mouse treated with mancozeb (400mg/kg/ day) showing vacuoles formed by exfoliation germ cells and disorganization of interstitial tissue and decreased in the number sperms in the lumen. HE X 200. Fig 4: Testis of the mouse treated with mancozeb (600mg/kg/ day) showing formation of giant cells resulting into reduced number of spermatogenic cells and lumen with loss of sperms. HE X 200. Fig 5: Testis of the mouse treated with mancozeb (800mg/kg/ day) showing formation of giant cells resulting in marked reduction in number of spermatogenic cells and sperms. Interstitial cells were also affected. HE X 200. Abbreviations: ST= Seminiferous tubules; EP= Epithelium; IN= Intertubular tissue, SG= Spermatogonia; SY= Spermatocytes; SM= Sperms. LU= Lumen.

Histologic studies of thyroid revealed that the treatment with 600 and 800 mg/kg/ day mancozeb caused decrease in the number of small follicles and increase in the number of medium, large follicles and total number of follicles significantly. However, treatment with 200 and 400 mg/kg/ day mancozeb showed no significant change in number of follicles (Table. 2). Histologic observations of the thyroid showed lumen fully packed with colloidal mass surrounding normal follicular cells (Fig.6).Histologic examination of thyroid with low dose of 200 mg/kg/ day mancozeb showed colloidal mass surrounded with distinct cuboidal follicular cells with initial hypertrophy (Fig. 7). Histological observations of the thyroid with increasing doses of 400,600 and 800 mg/kg/ day mancozeb revealed hypertrophy and hyperplasia of follicular cells with loss of colloid (Fig.8 to 10).

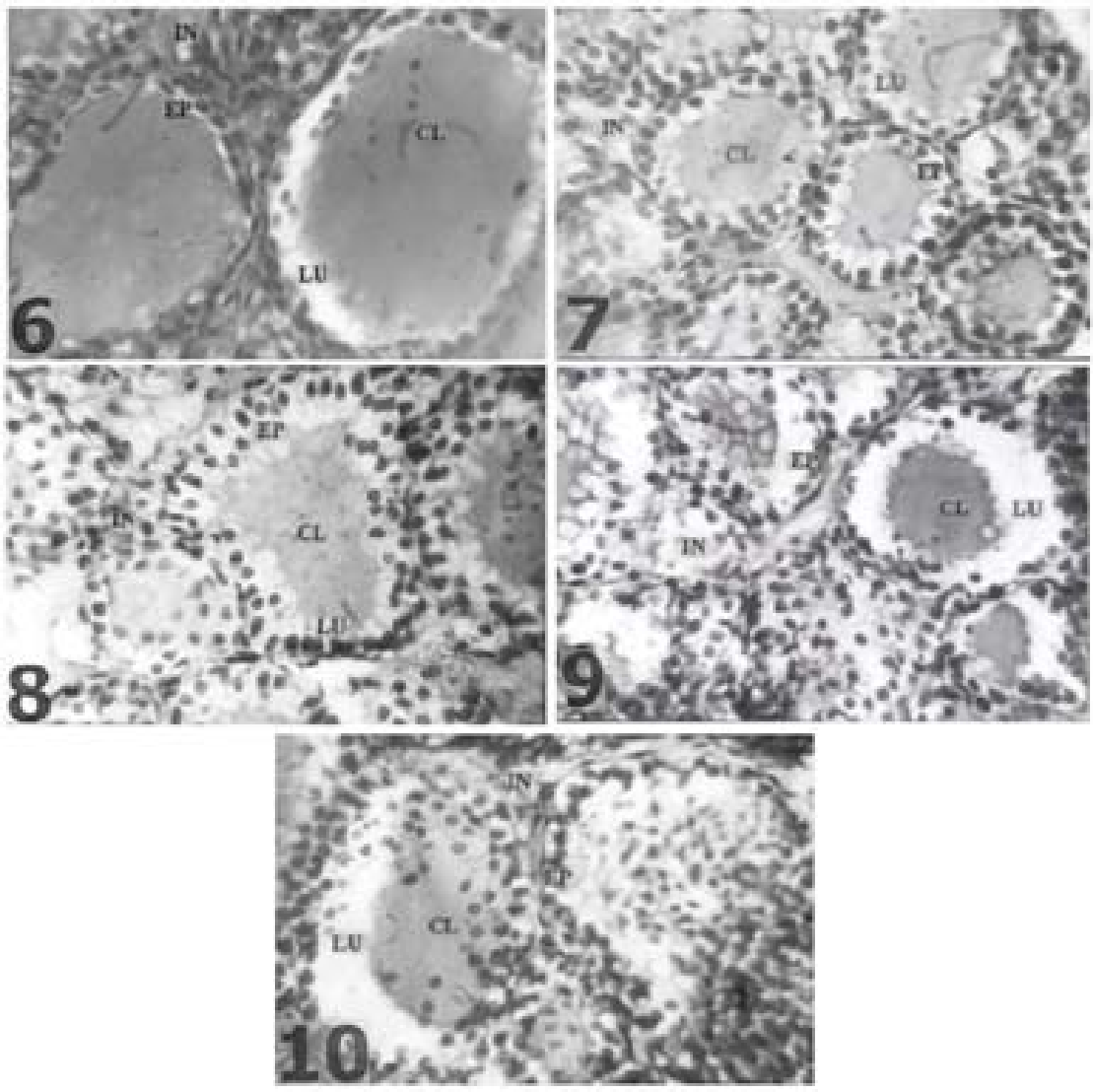

Fig 6: Thyroid of the vehicle-treated (control) mouse showing lumen fully packed with colloidal mass surrounding normal follicular cells. HE X 400. Fig 7: Thyroid of the the mouse treated with mancozeb (200mg/kg/ day) showing colloidal mass surrounded with distinct cuboidal follicular cells with initial hypertrophy. HE X 400. Fig 8: Thyroid of the mouse treated with mancozeb (400mg/kg/ day) showing lumen of follicles with decreased colloid mass and prominent hypertrophy of follicular cells. HE X 400. Fig 9: Thyroid of the mouse treated with mancozeb (600mg/kg/ day) showing follicles with loss of colloid mass with hypertrophy and hyperplasia of follicular cells. HE X 400. Fig 10: Thyroid of the mouse treated with mancozeb (800mg/kg/ day) showing hypertrophy and hyperplasia of follicular cells, presence of follicular cells in colloidal mass. HE X 400. Abbreviations: EP=Epithelium; C= Colloid, IN= Interstitium, LU= Lumen.

Table 1: Effect of mancozeb on weight of testes, number and diameter of spermatogonia, spermatocytes, spermatids and Leydig cells in albino mice

Dose (mg/kg/d)	Relative testes weight (mg/100g body wt; mean± SEM)	Number of spematogenic and leydig cells				Diameter (µm) of spermatogenic and leydig cells			
		Spermatogonia	Spermatocytes	Spermatids	Leydig cells	Spermatogonia	Spermatocyte	Spermatids	Leydigcells
Control	723.33 ± 0.25	74.17 ± 1.66	86.17 ± 2.80	151.33 ± 6.67	42.50 ± 3.71	7.75 ± 0.27	9.74 ± 0.30	6.00 ± 0.30	9.80 ± 1.87
200	688.35 ± 0.41	67.83 ± 1.49	80.50 ± 7.92	142.00 ± 12.57	40.33 ± 2.29	7.24 ± 0.46	9.24 ± 0.27	5.74 ± 0.41	8.94 ± 1.40
400	595.35 ± 0.27*	74.67 ± 3.40	75.67 ± 5.62	128.83 ± 9.20	35.33 ± 3.47	6.99 ± 0.17	9.01 ± 0.30	5.62 ± 0.42	7.60 ± 1.01
600	565.00 ± 0.41*	61.00 ± 1.93*	63.83 ± 0.67*	100.67 ± 7.67*	32.55 ± 2.17*	6.87 ± 0.16	8.79 ± 0.41	5.31 ± 0.35*	6.50 ± 0.76
800	556.66 ± 0.22*	59.83 ± 2.15*	59.83 ± 1.33*	98.83 ± 7.76*	31.33 ± 2.17*	6.49 ± 0.43*	7.74 ± 0.35*	4.82 ± 0.33*	5.41 ± 0.23*

Data are mean ±SE from six animals

*P < 0.05(When compared with control)

Table 2: Effect of mancozeb on thyroid weight and its follicular number in albino mice

Dose (mg/kg/d)	Wt. of thyroid (mg/100g)	Follicle size in µm(diameter)(mean ±SEM)			Total number of follicles
		Small (<30)	Medium (30 – 90)	Large (>90)	
Control	4.60 ± 0.01	62.16 ± 6.11	12.33 ± 1.81	5.33 ± 0.80	79.20 ± 5.56
200	5.88 ± 0.01	58.51 ± 6.36	18.50 ± 3.08	6.5 ± 0.95	82.83 ± 4.06
400	5.69 ± 0.01	53.66 ± 2.77	23.16 ± 3.14	8.50 ± 1.33	84.16 ± 3.24
600	7.87 ± 0.01*	41.33 ± 2.90*	39.43 ± 2.85*	13.16 ± 2.13*	93.41 ± 2.40*
800	8.46 ± 0.01*	39.46 ± 3.97*	45.33 ± 3.57*	14.16 ± 2.50*	98.33 ± 4.10*

Data are mean ±SE from six animals

* P < 0.05(When compared with control)

Table 3: Effect of mancozeb on organs weight in albino mice

Dose (mg/kg/d)	Relative organ weight / 100 g body wt (mean ± SEM)					
	Prostate gland (mg)	Cowper's gland (mg)	Kidney (g)	Spleen (mg)	Liver (g)	Thymus (mg)
Control	94.00 ± 0.14	152.30 ± 0.26	1.65 ±0.69	464.30 ± 0.44	5.83 ± 2.27	63.00 ± 0.07
200	85.60 ± 0.14	148.00 ± 0.18	1.62 ±0.65	427.66 ± 0.11	5.39 ± 2.03	60.48 ± 0.07
400	73.60 ± 0.16	127.75 ± 0.18	1.58 ± 0.44	407.83 ± 0.23	5.19 ± 2.90	81.92 ± 0.09
600	50.16 ± 0.24*	107.21 ± 0.26*	1.30 ± 0.50*	335.58 ± 0.47*	4.47 ± 3.29*	83.74 ± 0.06
800	45.21 ± 0.14*	101.80 ± 0.19*	1.26 ± 0.50*	327.00 ± 0.27*	4.29 ± 1.13*	102.11 ± 0.07*

Data are mean ±SE from six animals

* P < 0.05(When compared with control)

Behaviour, body and organs weight studies

The intoxicated mice were depressed and adopted abnormal posture with the head held in between the fore legs. They were trying to huddle at the corner of the cage and showed more running activity immediately after the administration of mancozeb. In the mice treated with 600 and 800 mg/kg/ day mancozeb showed significant decrease in the kidney, spleen, liver weight where as thyroid weight increased significantly. However, weight of these organs not changed significantly in the mice treated with mancozeb in other groups. Thymus weight increased significantly in the mice treated with600 and 800 mg/kg/ day mancozeb. However, thymus weight not changed significantly in the mice treated with mancozeb in other groups (Table 3). There was no significant change in bodyweight and weight of adrenal glands in all the mancozeb treated mcie compared with controls (data not shown).

Biochemical studies

In mice treated with 600 mg/kg/ day mancozeb showed significant decrease in the levels of glycogen and a significant increase in the level of total lipids in liver and a significant decrease in glycogen and total lipids in kidney. In mice treated with 800 mg/kg/ day mancozeb caused significant decrease in the level of protein in testis, liver and in kidney, glycogen in liver and kidney and a significant increase in the level of total lipids in testis. However, glycogen in testis and total lipids in liver and kidney not changed significantly. In the mice treated with mancozeb in other groups showed no significant changed in biochemical constituents of testis, liver and kidney when compared with those of the corresponding parameters of controls (Table4).

Table 4: Effect of mancozeb on biochemical constituents of the testis, liver and kidney in albino mice

Dose (mg/kg/d)	µg / mg wet weight of testis (mean ±SEM)			µg / mg wet weight of liver (mean ±SEM)			µg / mg wet weight of kidney (mean ±SEM)		
	Protein	Glycogen	Total lipids	Protein	Glycogen	Total lipids	Protein	Glycogen	Total lipid
Control	89.89 ± 0.14	6.95 ± 0.02	31.62 ± 0.17	187.25 ± 0.62	4.36 ± 0.05	32.26 ± 0.19	95.87 ± 0.20	3.93 ± 0.08	37.45 ± 0.02
200	85.07 ± 0.21	6.34 ± 0.08	34.78 ± 0.13	183.35 ± 0.51	4.04 ± 0.04	33.76± 0.40	91.25 ± 0.39	3.29 ± 0.09	34.5 ± 0.16
400	82.72 ± 0.18	6.18 ± 0.16	37.67 ± 0.19	178.51 ± 0.96	3.68 ± 0.06	35.97 ± 0.25	90.97 ± 0.17	3.00 ± 0.07	32.67 ± 0.08
600	80.89 ± 0.14*	6.10 ± 0.09	40.62 ± 0.16*	172.52± 0.71*	3.13 ± 0.05*	36.32 ± 0.20	88.47 ± 0.18*	2.78 ± .08*	30.65 ± 0.09
800	78.65 ± 0.15*	5.82 ± 0.08*	44.88 ± 0.15*	168.00± 1.29*	3.09 ± 0.05*	39.91 ± 0.17*	87.45 ± 0.21*	2.29 ± 0.09*	28.70±0.11*

Data are mean ±SE from six animals
* P < 0.05(When compared with control)

Discussion

Spermatogenic studies: Spermatogenesis is a process which involves the transformation of the undifferentiated germ cell in to highly differentiated immature spermatozoa/25/. Spermatogenesis involves inter play of sex steroid and pituitary gonadotropins/26/. Paired testicular mass, a valuable index of reproductive toxicity in male animals /27/decreased with increasing dose of pesticide and this decrease in testicular mass was consistent with elimination of germ cells /28/. In the present study weight of testes decreased significantly with increasing doses of pesticide. Similar effects have been observed that the testicular atrophy with damaged germinal epithelium and reduced sperm motility and viability were major findings in male adult rats exposed to maneb and zineb /29/. It has been observed that carbaryl induce sperm abnormalities, reduced number of spermatogonia and spermatozoa in mice and rats /9/. It has been showed that the treatment with a carbamate insecticide carbaryl affects spermatogenic cells and causes Leydig cells degeneration and alters the testosterone and gonadotrophin levels in blood serum, testicular total lipid and alkaline acid phosphatase activity /30/. It has been reported that mancozeb shows it's biological effects through their metabolites like ethylene thiourea (ETU) and carbon disulphide (CS2) /1/.The carbon disulphide causes significant decrease in serum testosterene, marked degenerative changes in

testicular tissue, affects spermatogenesis and also epididymal alterations /2/.

In the present study the effect of mancozeb on testis and accessory sex organs revealed two principal impacts on the male reproductive system of mice namely, the anti spermatogenic and anti-androgenic effects. The anti- spermatogenic adverse effect is reflected by the decrease in number of spermatogenic cells. The defective seminiferous tubules found more in the testis of treated mice and rarely in the testis of control mice. The appearance of defective seminiferous tubules may be due to testicular apoptosis. Growth factors and cytokines are also involved in local control mechanism influencing testicular apoptosis through paracrine and autocrine mechanisms. Intra testicular androgens, secreted by Leydig cells, also play an important paracrine role in preventing germ cell degeneration /31,32/.The elevation of testicular temperature or other change associated with cryptorchidism may cause germ cell apoptosis /33/. Apoptosis is a physiological process of cell death leading to the controlled elimination of single unwanted cell from the midst of a viable tissue without damaging the neighbouring unaffected cells /34,35/. In the present study the observed defective seminiferous tubules posses decreased spermatogenic cells and more giant cells with loss of sperms in the lumen. Formation of giant cells were reported in rats treated with carbamate fungicide Zineb /36/. Similarly the organophosphate insecticide Phosphomidon also known to cause formation of giant cells in the rats at a dose of 35 ppm orally/37/. Phopshomidon induces chromatin and chromosome breaks, dot deletions, fragmentation and anaphase bridges in cells engaged in mitotic division and fragmentation, anaphase bridge and laggards in cells engaged in meiosis /38,39,40/. Such chromatin and chromosomal damage may correlate with inhibition of DNA synthesis, failure of chromosomal replication or failure of the cell to divide into daughter cells after the nucleus has divided leads to formation of giant cells. Severe spermatogenic inhibition has been reported by Carter et al.,/41/ in the rats treated with a carbamate fungicide Carbendazim(400mg/kg/ day) for 10 days .Histological examination of testis 245 days post exposure revealed severe seminiferous tubular atrophy (>85%).These seminiferous tubules showed Sertoli cells only with thickened basement membrane. Once a tubular basement membrane has thickened , that portion of the tubule may no longer be available for normal spermatogenesis. The anti-androgenic action of mancozeb in the present study possibly reflected decrease in the weight of prostate and Cowper's glands. The accessory male ducts and glands are morphologically and physiologically dependent upon the production of androgens /42/.The present study is comparable to the findings made by Samuel et al., /43/ that reduced testicular weight and maturational arrest of the primary spermatocytes manifests androgen deficiency. Therefore, the toxicants that interfere with testis function could do so indirectly by acting at the level hypothalamus or pituitary gland or both /44/. It has been observed that members of carbamate pesticides such as disulfiram and its metabolite dithiocarbamate, can interfere with catecholamine neurotransmitter metabolism by inhibiting the activity of dopamine β-hydroxylase (DβH), this is an enzyme that converts dopamine to norepinephrine and the norepinephrine then stimulates the release of GnRH. Thus GnRH release is affected through the inhibition of DβH /45, 46/. This mechanism plays an important regulatory and /or modulatory role in brain hypothalamic control of pituitary luteinizing hormone (LH) release/47/. In rats administration of *N* -methyl dithio carbamate causes suppression of LH surge by interfering with catecholamine activity /6/.In the present study this could be expected to affect gonadal steroidogenesis and spermatogenesis with mancozeb treatment.

Vital organ studies: In the present study exposure to increasing doses of mancozeb resulted in a significant decrease in the weight of liver, kidneys and spleen. Similar findings have been suggested that administration of mancozeb with high dose (1500 mg/kg/ day) and chronic exposure (360 days) causes sign of poisoning, decrease in kidney weight and pathomorphological changes in liver, brain and kidney in rats/48/. In the present study decreased organs weight by mancozeb exposure may be due to accumulation of its substitutes in the tissues. Mancozeb is an ethylene bis dithiocarbamate fungicide consists of ethylene bis dithiocarbamate ion ,58.13%, manganese++ 15%, zinc++1.87%, inert ingredients 25% /49/. Manganese concentrate in mitochondria so that tissues rich in these organelles have highest concentration of manganese including pancreas, liver, kidney, and intestine. Biological half life of manganese in the body is 37 days, if readily crosses the blood brain barrier and half life period in the brain is longer than in the whole body. Zinc concentration in tissues varies widely. Liver receives up to 40% of dose. Liver concentration is influenced by humoral factor including adrenocorticotropic hormones, parathyroid hormones. In the liver and other tissues zinc is bound to metallothionein. The greatest concentration of zinc in the body is in the prostate; probably releated to the rich content of zinc, containing enzyme acid phosphatase/50/ .The condition may be same with other organs resulting in decrease in their weight. Present study with mancozeb revealed there was significant decrease in spleen weight and significant increase in thymus weights indicates sign of immunosuppression. Spleen is the site of extramedullary erythropoiesis and removal of damaged

blood cells, spleen is the major filter of blood borne antigens including toxicants bound with serum proteins.Pesticide interference with spleen metabolism resulted in decreased spleen weight. Mancozeb caused thymus enlargement in higher doses.This lymphoproliferative response might be to recruit new cells to over come the immunosupression caused by the toxicant.

Thyroid effect studies: Thyroid regulates male reproduction by maintaining gonadal homeostasis and adjuvating the action of gonadotropins. Thyroid hormone defieciency may be associated with morphological and functional alterations in pituitary testicular hormonal axis and there by the structure and function of testis and accessory sex organs is also altered /51/. Thyroidectomy in immature male rats caused severe inhibition of gametogenesis and leydig cell development /52, 53/. Thyroid hormones have supportive role in cell metabolism in the testis. In adult male rats hypothyroidism induced by thyroidectomy or goiterogen treatement was found to cause degenerative changes in testis/54/. In the present study thyroid weight increased significantly by treatment with mancozeb in dose dependent manner.Similar effect have been reported with mancozeb treated rats/3/. Increased thyroid weight may be the result of direct action of mancozeb on thyroid by inhibiting thyroxine synthesis and accelerates its deiodination and causes increased pituitary TSH levels. Increase in thyroid weight was likely to be due to increase in circulating thyroid stimulating hormone/55/. Administration of anti thyroid chemical causes the deficiency of thyroxine synthesis as a result low level of circulating thyroxine is observed.This low level of thyroxine results in the increase out put of TSH by antirior pituitaty which causes the hypertrophy and hyper plasia of thyroid epithelium.During pathogenic conditions the amount of colloid secreted by the follicles fluctuates and when the thyroid is inactive state colloid accumulates in the epithelial cells and the cells become low cuboidal or squamous /56/.

Jannini et al.,/57/ have reported in the rats chronic hypothyroidism is induced by methimazole treatment from birth to adult hood delays cessation of Sertoli cell proliferative activity. Absence of the differential effect of T3 delays the appearance of the tubular lumen leading to a reduced final testicular size. The same investigators found the presence of high affinity - low capacity thyroid hormone receptor sites in fertile neonatal and at a lower level, in pre pubertal but not in adult testis of rat. The testes are responsive to thyroid hormone only during a limited period of time coinciding with perinatal and peripubertal stages. The adult male gonad is unresponsive to thyroid hormone. But it has been found that the rams hypothyroidism results in reduced testosterone secretory Leydig cells /52/. Jiyang et al., /14/have reported thyroxine treatment to the infertile *rdw* rats caused recovery in testicular and epididymal weights with increased serum T4 levels. The sexual behaviour testicular and epididymal function brought to normal. Complete reversion was determined both invitro and invivo for fertilization without loss of sperm viability. Ethylene thio urea (ETU) and carbon disulfide (CS2) are the major metabolites of mancozeb known to cause increased thyroid weight in the rat /1/. Because ETU is relatively accumulates in thyroid, irrespective of the way of exposure and thyroid gland is most affected organ /4/. The major source for ETU is ethylene bisdithiocarbamateion an active ingredient of mancozeb which comprises 58.13% /50/. In the present study thyroid toxicity might be one of the consequence to affect testicular homeostasis.

Proteins, carbohydrates and lipids are essential constituents of the food of animals. Proteins are the building blocks, carbohydrates are the immediate source of energy and lipids are reservoirs of energy. The data obtained in the present study revealed the levels of protein glycogen and total lipids in testis, liver and kidney were significantly not changed with low dose treatment. However, treatement with high dose caused significant decrease in the level of protein and glycogen and significant increase in total lipids in testis and liver. Where as in kidney all biochemical constituents decreased significantly. It has been reported that lindane inhibits testicular steroidogenic enzymes, testicular DNA, RNA and proteins and affects male reproduction /58/. It has been showed that diethyl dithiocarbamate inhibits hepatic cyt P-450 dependent enzyme activity in rats /59/. It has been suggested that there was a significant decrease in the levels of blood glucose and globulin in mancozeb treated rats, due to low thyroxine level because of impaired thyroid function /60/.It has been found that increase in the levels of phosphoinositoides and phosphotidic acid in liver suggest the likely involvement of phospholipase c-path way of signaling in the toxicity of mancozeb in different tissues at varying levels /61/.In conclusion the results of the present study indicate that the observed effects of mancozeb on thyroid and testis may be due to interference of compound resulting into impaired thyroid- testis interrelationship, leading to the infertility of exposed population. These observed effects of mancozeb on testis, thyroid and biochemical constituents may be due to hormonal imbalance in any of the stages in the hypothalamo-hypophysialtesticular axis or hypothalamo – hypophysial –thyroid axis. Further investigations are needed to determine whether the increased incidence of thyroid effects leads to direct effect on testis or the effect is mediated via some other endocrinological factor(s). The changes in the levels of protein, glycogen and total lipids with mancozeb treatment suggest either an increased catabolism of the biomolecules to meet the enhanced energy

demand of animals under stress or their reduced synthesis due to impaired tissue function Mancozeb though having acute mammalian toxicity exhibit significant toxicological effects after repeated chronic exposure.

Acknowledgement

We thank Chairman Post-graduate department of Zoology, Karnatak University, Dharwad for providing research facilities.

References

1. O'Neil. WM, Marshal WD. Goitrogenic effect of the etuylene thiourea on rat thyroid .Pestic Biochem Physiol.1984; 21: 92-101
2. Patel Kumud GA, Gautam K, Vaghasia YV. Carbon disulphide induced impairments in male reproductive system in rats. Ind J Physiol All Sci .1999;53: (1), 22- 28
3. Trivedi N, Kackar R, Srivastava MK, Mithal A, Raizada RB. Effect of oral administration of fungicide mancozeb on thyroid gland of rat. Ind J Expt Biol .1993;31:564-630
4. NTP Technical reports on the perinatal toxicology and carcinogenesis studies of ethylene thiourea in T344/N rats and B6C3F mice. 1992.US Depatment Of Health And Human Services.
5. U.S. Environmental Protection Agency. Guidance for the Registration of Pesticide Products Containing Maneb as theActive Ingredient. Washington, DC, 1988; 4-11.
6. Goldman JM, Stocker JE, Cooper RL, McElory WK, Hein JE. Blockade of ovulation in the rat by fungicide sodium N-methyl dithiocarbamate relationship between effects on the luteinizing hormone surge and alterations in hypothalamic catecholamines. Neurotoxicology and Teratology. 1994; 16: 257-268
7. Nakai MI, Hess RA. Effects of carbendazim (methyl-2-benzimidazole carbamate MBC) on meiotic spermatocytes and subsequent spermatogenesis in the rat testis. Anat Rec.1997; 247(3): 379-387.
8. Matsuo-Fusako, Nakai-Masaaki, Nasu –Testsuo. the fungicide carbendazim induces meiotic micronucleus. J Vet med sci .1999;61 (5): 573-576.
9. Kitagawa K, Wa Ka, Kura M, Ishikawa S. Light microscope study of endocrine organs of rats treated by carbamate pesticides .J Toxicol Sci .1977;2: 53-60.
10. Kid GS, Glass AR, Vigersky RA. The hypothalamic pituitary testicular axis in thyrotoxicosis. J Clin Endocrinol Metab.1979; 48: 798-802.
11. Worstman. J, Rosner W,Dufau ML. Abnormal testicular function in men with primary hypothyroidism. Am J Med .1987; 82,207-212.
12. Jayakumar B,Khurana ML,Ammini AL, Karmarkar MG, Ahuja MMS . Reproductive endocrine functions in men with primary hypothyroidism : effect on thyroxine replacement. Hum Res .1990 ;34:215-218.
13. Beamer NG, Eicher EM, Maltais LJ, Southard JL. Inherited primary hypothyroidism in mice.Science, 1981; 212; 61-63.
14. Jiyang JY, Umezu M, Sato E.Characteristics of infertility and improvement of fertility by thyroxine treatment in adult male hypothyroid *rdw* rats.Biol Reprod 2000;63:1637-1641.
15. Hui QV, Armstrong C, Laver G. Mono oxygenase mediated metabolism and binding of ethylene thiourea to mouse liver microsomal protein. Toxicol lett. 1988; 41:231-237.
16. Leblond CP, ClermontY. Definition of the stages of the cycle of the seminiferous epithelium in rat.Ann.NY.Acad.Sci 1952; 55:548-573.
17. Oakberg EF. Duration of spermatogenesis in mouse and timing of the stages of the cycle of seminiferous epithelium. Am.J.Anat 1956; 99,507-516.
18. Gardner P. Fine structure of seminiferous epithelium of the swiss mouse.The spermatid.Anat.Res 1966;155,235-250.
19. Dym M, Fawcett DW. Further observations of the number of spermatogonia, spermatocytes and spermatids connected by inter cellular bridges in mammalian testis.Biol.Reprod. 1971;4,195-215.
20. Fawcett DW, Neaves WR, Flores MN. Comparitive observations on intertubular lymphatic and the organization of the interstitial tissue of mammalian testis. Biol.Reprod 1973;9 :500-532.
21. deKrester DM, Kerr JB. The cytology of the testis. In :Knobil.E.,Neil.J.D., (Eds).The physiology of reproduction. Vol.2.Raven Press.1994; New York. pp 1177- 1240.
22. Lowry H, Rosebrough NI, Far AL. Ranall RJ. Protein measurement with folinphenol reagent. J. Biol. Chem. 1951; 193: 265-275.
23. Scieffer S, Dayton S, Novic B, Myntiyer E. The intimation of glycogen with the anthrone reagent .Arch Biochem 1950 ;25:191.
24. Folch J, Leu M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue .J Biol Chem 1957; 226: 497-507
25. Clermont Y. Kinetics of spermatogenesis in mammals seminiferous epithelium cycle and spermatogonial renewal. Physiol Rev.1972 ;52:198-265.
26. Sharpe RM. Testosterone and spermatogenesis. J. Endocrinol. 1987;113:1.

27. Amman RP. A critical review of methods for evaluation of spermatogenesis from seminal characeteristics. J Androl.1982; 2: 37-38.
28. Chapin RE, Lamb JC. Effects of ethyleneglycolmonoethylether on various parameters of testicular function in the F344 rats.Environ Health Persp.1984; 57;219-224.
29. Lucier GW, Lee IP, Dixon RL. Effects of environmental agents on male reproduction. In "The testis" vol-IV. Eds. By Johnson AD, Grames WR, 577 Academic Press, 1977; New York.
30. Shrivastava SM, Shrivastava VK. Toxicological effects of carbaryl on testicular morphology and testosterone levels in mus musculus. Poll Res .1998;17(3): 215-8.
31. Billig.H, Chun SY, Eisenhauer K, Husueh AJW. Gonadal cell apoptosis; hormone-regulated cell demise. Hum Reprod .1996;2: 103-117.
32. Tapanainen JS, Tilly JL, Vinko KK, Hsueh AJ. Hormonal control of apoptic cell death in the testis, gonadotropins and androgens as testicular cell survival factors. Mol Endocrinol. 1993; 7; 643-650.
33. Yin Y, Dewolf WC, Morgentaier A. Experimental cryptorchidism induces testicular germ cell apoptosis by *p*53-dependent and independent pathways in mice. Biol Reprod .1998;58: 492-496.
34. Pesce M, Farrace MG, Piacentini M, Dolci S, Felci MD. Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death(apoptosis). Development. 1993;11: 1089-1094.
35. Averal HI., Stanley A, Akbarsha MA. Apoptic death of male germinal line cells of rat caused by Vincristine: light microscopic and electron microscopic study. Biomed Lett . 1996;52:171-180.
36. Raizada RB, Dutta KK, Dikshit TSS. Effects of Zineb on male rats.Bu ll Environ Contam Toxicol 1979;22: 208-213.
37. Akabarsha MA, Sivasamy P. Apoptosis in male germinal line cells of rat in vivo: caused by Phosphomidon. Cytobios. 1997;91:33-44.
38. Reddy MV, Rao BVR. The cytological effects of insecticides (Dimecron-100 and Roger-40) on Vicia faba L.Cytologia .1969;34:410-417.
39. Georgian L.The comparative cytogenic effects of aldrin and Phophomidon. Mut Res 1975;31: 103-108.
40. Usharani MV, Reddy PP. Mutagenic effects of Dimethoate and Phosphomidon in mice by host – mediated assay. Cell Chr Res .1986:9: 36-38.
41. Carter SD, Hess RA, Laskey JW. The fungicide methyl 2-Benzimidazole carbamate causes infertility in male Sprague-dowley rats. Biol. Reprod.1987; 37: 709-717.
42. Williams-Ashman HG, Reddy AH. Androgenic regulation of tissue and function. In the Biochemical actions of hormones Vol II Eds G Litwack. 1972; Academic Press, New York.
43. Samuel LT,Shoot JG, Huseby RA. The effect of diethylstilbesterol on testicular hydroxylase and 17 α – desmolase activities in BALB/c mice.Acta. Endocrinol.1964;45:487.
44. Smith. CG. Reproductive toxicity: Hypothalamic pituitary mechanisms. Am J Indust Med .1983; 4:107-112.
45. Maj J, Vetulani J. Effect of some N, N-disubstituted dithiocarbamates on catecholamines level in rat brain. Biochem. Pharmacol. 1969;18:2045-2047.
46. Przewlocka B, Sarnek J, Szmiielski A, Niewiakomsha A. The effect of some dithiocarbamic acids on dopamine-β-hydroxylase and catecholamines level in rats brain. Pol. J. Pharmacol. Pharm. 1975; 27:555-559.
47. Kalra SP, Kalra PS. Neutral regulation of luteinizing hormone secretion in the rat. Endocr. Rev. 1983;4:311-351.
48. Kackar R, Srivastava M.K, Raizada RB. Assessment of toxicological effects of mancozeb in male rats after chronic exposure. Ind. J. Expt. Biol.1999; 37: 553-559.
49. Mancozeb pesticide fact sheet.1995. http: // infoventures.com/e-hlth/
50. Robert AG. Toxic effects of metals..In Curtis D, Klaassen, Mary O, Amder, John Doull(Eds) Casarett and Doull's Toxicology. 1986.Mac millan publishing company NY.pp 582-635.
51. Chandrashekhar Y, Holland MK, D'Occhio MJ, Setchell BP. Spermatogenesis, seminal charecteistics and reproductive hormone levels in mature rams with induced hypothyroidism and hyper thyroidism.J Endocr .1985;105:,39-46.
52. Chowdury AR, Arora O. Role of thyroid in testicular development of immature rat.Arch Androl .1984 ;12:49-51.
53. Chowdury AR, Gautam AK, Chatterjee BB. Thyroid testis interrelationship during the development and sexual maturity of the rat.Arch Androl 1984;13:233-239
54. Amin SO, Sheikh AS. Pituitary testicular function in hypothyroid male rats.Acta Anat (Basel) 1977; 98:121-129
55. Ivanova-Chemishanska L, Markov DV, Deshev G. Light and electron microscopic observation on rat thyroid after administration of some dithiocarbamates .Environ Res 1971; 4: 201-212.
56. Turner CD, Bagnar JT. General endocrinology. 1971. W.B.Saunders.Philadelphia.
57. Jannini EA, Ulisse SD, Armiento M.Thyroid hormone and male gonadal function. Endocr Rev .1995;16:443-459.
58. Sujatha R, Chitra KC, Latchoumycandave C, Mathur PP. Effect of lindane on testicular anti oxidant system and stroidogenic enzymes in adult rats. Asian J. Androl. 2001;3: 135-138.

59. Stott, Ian, Anupama Murthy, Alex Robinson, Norman .W., Thomas, Jeffrey. R., Fry. Low dose diethiocarbamate attenuates the hepatotoxicity of 1-3-dichloro-2- proponol and selectively inhibits CYP 2E1 activity in the rat .Human Expt Toxicol .1997; 16(5): 262-266.
60. Nebbia C, Ferrero E. Pathologic changes tissue distribution and extent of common to ETU after sub acute administrations of Zinc ethylene bisdithiocarbamate to calves with immature resmen function. Am J Vet Res .1991;52: 1717-1722.
61. Subramoniam A, Deepa Agrawal, Srivistava SP, Seth PK. Influence of mancozeb on mitogenically responsive lipids in rat cerbebrum and liver. Ind J Expt.Biol.1991;29;943-945.