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

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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 whereas 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, whereas 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 excreted 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 lutentizing 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 aberration 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

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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 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±1°C), and light (12 h 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 mucus 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 with 600 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.](image1)

![Fig 2: Testis of the mouse treated with mancozeb (200mg/kg/day) showing normal spermatogenesis. HE X 200.](image2)

![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.](image3)

![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.](image4)

![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.](image5)
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

<table>
<thead>
<tr>
<th>Dose (mg/kg/d)</th>
<th>Relative testes weight (mg/100 g body wt, mean ± SEM)</th>
<th>Number of spermatogenic and leydig cells</th>
<th>Diameter (µm) of spermatogenic and leydig cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spermatogonia</td>
<td>Spermatocytes</td>
</tr>
<tr>
<td>Control</td>
<td>723.33 ± 0.25</td>
<td>74.17 ± 1.66</td>
<td>86.17 ± 2.80</td>
</tr>
<tr>
<td>200</td>
<td>683.35 ± 0.41</td>
<td>67.83 ± 1.49</td>
<td>80.50 ± 7.92</td>
</tr>
<tr>
<td>400</td>
<td>595.35 ± 0.27*</td>
<td>74.67 ± 3.40</td>
<td>75.67 ± 5.62</td>
</tr>
<tr>
<td>600</td>
<td>565.00 ± 0.41*</td>
<td>61.00 ± 1.93*</td>
<td>63.83 ± 0.67*</td>
</tr>
<tr>
<td>800</td>
<td>556.66 ± 0.22*</td>
<td>59.83 ± 2.15*</td>
<td>59.83 ± 1.33*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Dose (mg/kg/d)</th>
<th>Wt. of thyroid (mg/100g)</th>
<th>Follicle size in µm(diameter)(mean ±SEM)</th>
<th>Total number of follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Small (&lt;30)</td>
<td>Medium (30 – 90)</td>
</tr>
<tr>
<td>Control</td>
<td>4.60 ± 0.01</td>
<td>62.16 ± 6.11</td>
<td>12.33 ± 1.81</td>
</tr>
<tr>
<td>200</td>
<td>5.88 ± 0.01</td>
<td>58.51 ± 6.36</td>
<td>18.50 ± 3.08</td>
</tr>
<tr>
<td>400</td>
<td>5.69 ± 0.01</td>
<td>53.66 ± 2.77</td>
<td>23.16 ± 3.14</td>
</tr>
<tr>
<td>600</td>
<td>7.87 ± 0.01*</td>
<td>41.33 ± 2.90*</td>
<td>39.43 ± 2.85*</td>
</tr>
<tr>
<td>800</td>
<td>8.46 ± 0.01*</td>
<td>39.46 ± 3.97*</td>
<td>45.33 ± 3.57*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Dose (mg/kg/d)</th>
<th>Relative organ weight / 100 g body wt (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prostate gland (mg)</td>
</tr>
<tr>
<td>Control</td>
<td>94.00 ± 0.14</td>
</tr>
<tr>
<td>200</td>
<td>85.60 ± 0.14</td>
</tr>
<tr>
<td>400</td>
<td>73.60 ± 0.16</td>
</tr>
<tr>
<td>600</td>
<td>50.16 ± 0.24*</td>
</tr>
<tr>
<td>800</td>
<td>45.21 ± 0.14*</td>
</tr>
</tbody>
</table>

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 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 with 600 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 mice 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 (Table 4).

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

<table>
<thead>
<tr>
<th>Dose (mg/kg/d)</th>
<th>µg / mg wet weight of testis (mean ±SEM)</th>
<th>µg / mg wet weight of liver (mean ±SEM)</th>
<th>µg / mg wet weight of kidney (mean ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>Glycogen</td>
<td>Total lipids</td>
</tr>
<tr>
<td>Control</td>
<td>89.89 ± 0.14</td>
<td>6.95 ± 0.02</td>
<td>31.62 ± 0.17</td>
</tr>
<tr>
<td>200</td>
<td>85.07 ± 0.21</td>
<td>6.34 ± 0.08</td>
<td>34.78 ± 0.13</td>
</tr>
<tr>
<td>400</td>
<td>82.72 ± 0.18</td>
<td>6.18 ± 0.08</td>
<td>37.67 ± 0.13</td>
</tr>
<tr>
<td>600</td>
<td>80.89 ± 0.14*</td>
<td>6.10 ± 0.09*</td>
<td>40.62 ± 0.16*</td>
</tr>
<tr>
<td>800</td>
<td>78.65 ± 0.15*</td>
<td>5.82 ± 0.08*</td>
<td>44.88 ± 0.15*</td>
</tr>
</tbody>
</table>

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 spermatooza/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 spermatooza 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 testosterone, 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/. Phosphomidon 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 (DBH), 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 DPH /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 /5/. 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 related 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 deficiency 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. Thyroidectomy in immature male rats caused severe inhibition of gametogenesis and Leydig cell development. Thyroid hormones have supportive role in cell metabolism in the tests. In adult male rats hypothyroidism induced by thyroidectomy or goitrogen treatment was found to cause degenerative changes in testis. 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. 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. 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 anterior pituitary which causes the hypertrophy and hyperplasia 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.

Jannini et al. 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. Jiyang et al., have reported thyroxine treatment to the infertile rats caused recovery in testicular and epididyimal weights with increased serum T4 levels. The sexual behaviour testicular and epididyimal function brought to normal. Complete reversion was determined both in vitro and in vivo for fertilization without loss of sperm viability. Ethylene thiourea (ETU) and carbon disulfide (CS2) are the major metabolites of mancozeb known to cause increased thyroid weight in the rat. Because ETU is relatively accumulates in thyroid, irrespective of the way of exposure and thyroid gland is most affected organ. The major source for ETU is ethylene bisdithiocarbamate an active ingredient of mancozeb which comprises 58.13%. 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, treatment 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. It has been showed that diethyl dithiocarbamate inhibits hepatic cyt P-450 dependent enzyme activity in rats. 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. 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.

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-hypophysial-testicular axis or hypothalamo – hypophysial –thyrroid 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.

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