

Induction of Gonadal Toxicity to Female Rats after Chronic Exposure to Mancozeb

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Abstract: Mancozeb, a fungicide of ethylenebisdithiocarbamate group was orally administered at doses of 500, 600, 700 and 800 mg/kg body weight/day to normal virgin rats of Wistar strain for 30 days. The vaginal smear and body weight of the rats were recorded daily and rats were sacrificed on 31st day. Estrous cycle was effected by showing a significant decrease in the number of estrous cycle, duration of proestrus, estrus and metestrus with concomitant significant increase in the duration of diestrus in all the mancozeb treated groups when compared with controls. There were a significant decrease in the number of healthy follicles and a significant increase in the number of atretic follicles in all the mancozeb treated groups when compared with controls. The histologic observation of the ovary revealed the presence of less number of corpora lutea and the size of the ovary was also reduced in high doses of mancozeb treated rats. There was a significant increase in the thyroid weight in all the mancozeb treated rats except in 500 mg/kg/d. In rats treated with 500 mg/kg/d showed a significant increase in the level of total lipids in the liver. In rats treated with 600 mg/kg/d mancozeb showed a significant decrease in the levels of glycogen and total lipids in the uterus and total lipids in the liver. In rats treated with 700 mg/kg/d showed a significant decrease in the levels of protein in ovary, glycogen, total lipids, phospholipids and neutral lipids in the uterus and a significant increase in the levels of phospholipids, neutral lipids in the ovary and total lipids, phospholipids and neutral lipids in the liver. In rats treated with 800 mg/kg/d showed a significant decrease in the levels of protein and glycogen in the ovary and protein, glycogen, total lipids, phospholipids and neutral lipids in the uterus and a significant increase in the levels of total lipids, phospholipids and neutral lipids in the ovary and liver when compared with controls. These observed effect of mancozeb on the estrous cycle, follicles and biochemical constituents may be due to imbalance in the hormone or toxic effect.

Key words: Mancozeb, Ovary, Follicles, Estrous cycle, Female rats, Biochemical constituents, Toxicity

Introduction

Mancozeb is a polymeric complex of zinc and manganese salts of ethylenebisdithiocarbamate (EBDC). It is commonly used for foliar application and seed-treatment in agriculture. Mancozeb is greyish-yellow powder that is stable under normal storage condition, but decomposes at high temperature due to moisture and acid¹⁾. Despite its low acute toxicity mancozeb has been shown to produce significant

toxicological effects on thyroid, gonads in male rats and chromosomes of bone marrow cells in mice^{2,3)}. Exposure to mancozeb causes normocytic type of anaemia, significant decrease in blood glucose and globulin levels and a significant pathological changes were observed in liver, kidney, spleen and heart showed congestion with slight enlargement and brain revealed few petechial hemorrhage⁴⁾. Mehrotra *et al.*⁵⁾ have reported that the mancozeb is regarded as weak mutagen and has some carcinogenic activity in mouse. It has been reported that the administration of a fungicide sodium N-Methyl dithiocarbamate inhibits the secretion of lutenizing

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hormone thus affecting ovulation in rat⁶.

In the view of the fewness of information on female reproductive system, the present study has been undertaken to know the effect of mancozeb on estrous cycle, morphometric analysis of follicular growth and biochemical constituents in virgin albino rats.

Materials and Methods

Mancozeb (commercial grade of 75% wettable powder) was made available from Indofil Chemicals Company, Mumbai and dissolved in olive oil for oral administration. Doses were given according to the body weight of rats per day. Virgin female rats of Wistar strain (12–17 weeks old) weighing between 150–170 g, showing regular estrous cycle length (4–5 days) were selected randomly and kept in individual cages with 12:12 hr light and dark cycle and at room temperature of 26 ± 10 C. Animals were given synthetic pellet diet “Gold Mohar” (Hindustan Lever Company, Mumbai) and water provided *ad libitum* throughout the study. Animals were divided into 5 groups having 8 rats in each. Mancozeb in doses of 500, 600, 700 and 800 mg/kg/d was administered orally for 30 d to respective groups. The rats of control group were given an equivalent volume of olive oil. Daily vaginal smear and body weight were recorded throughout the experiment to find out the per cent change. The phases of estrous cycle were determined by observing the vaginal smear in the morning (0800 h to 1000 h) as described by zarrow *et al.*⁷. All animals were sacrificed by cervical dislocation on the 31st day, 24 h after the final exposure and soon after the last vaginal smear.

Morphometric analysis of follicular growth studies

Ovaries of four animals were taken for follicular growth studies. The weight of the ovaries of the animal nearest to the mean weight of the ovaries of the respective group was selected. The ovaries were fixed in Bouin’s fluid, embedded in paraffin, sectioned at 5 μ m thickness and stained with hematoxylin and eosin. Sections of the ovary were examined under the light microscope and the general histologic appearance was assessed. In addition, all the serial sections were examined for the presence of different stages of healthy follicles, described by Hirshfield and Midgley⁸, based on their diameter. Follicles displaying the nuclei of oocytes were measured using a calibrated ocular micrometer, to avoid repeated counting. The maximum diameter and diameter at right angles to it were used to obtain a mean diameter. A follicle was considered undergoing atresia or regressing whenever two or more pyknotic granulosa cells could be

found in a single section or whenever the oocyte showed obvious signs of degeneration such as fragmentation, loss of nuclear membrane or thinning of the cumulus oophorus, as proposed by Osman⁹. These atretic follicles were also counted in each section of the ovary and categorised along with the healthy follicles based on the diameter.

The per cent increase in body weight was calculated on the basis of the weight taken soon after the oral administration considered as the initial body weight and the weight taken on 31st d before cervical dislocation was considered as the final body weight. Ovary, uterus, kidney, adrenal, spleen, liver, lungs, heart, thymus and thyroid were dissected out, freed from adherent tissue and weighed to the nearest milligram. To ensure normalization of data for statistical analysis, organs weight were expressed per 100 g body weight.

Biochemical studies

Freshly removed ovary, uterus and liver tissues were weighed to required milligram for biochemical analysis such as protein, glycogen, total lipids, phospholipids and neutral lipids. The net weight of the tissues were estimated gravimetrically. Protein estimation was performed as per the method described by Lowry *et al.*¹⁰ glycogen by Sciefter *et al.*¹¹ total lipids, phospholipids and neutral lipids by Folch *et al.*¹². Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett’s test ($P < 0.05$).

Results

Estrous cycle studies

The control rats exhibited regular estrous cycle and normal duration of each phase of estrous cycle. Treatment with mancozeb in all doses caused a significant decrease in the number of estrous cycle and duration of proestrus, estrus and metestrus with concomitant significant increase in the duration of diestrus phase. Diestrus index was also increased dose-dependently following the administration of mancozeb treatment. Change in estrous cycle was observed first in the highest dose i.e. 800 mg/kg/d and was gradually observed in lower doses (Table 1). However, the intoxicated rats were depressed and adopted abnormal posture with forward placing of both fore and hind limbs 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.

Table 1. Effect of mancozeb on estrous cycle in albino rats

| Groups | Treatment (mg/kg/d) | Number of rats | Number of cycles | Duration in days (M ± S.E.) | | | | Diestrus index |
|--------|---------------------|----------------|------------------|-----------------------------|--------------|--------------|---------------|----------------|
| | | | | Proestrus | Estrus | Metestrus | Diestrus | |
| I | Control + oil | 8 | 5.71 ± 0.18 | 5.00 ± 0.22 | 7.71 ± 0.29 | 4.86 ± 0.26 | 12.29 ± 0.42 | 40.95 |
| II | 500 | 8 | 3.57 ± 0.20* | 2.43 ± 0.20* | 4.71 ± 0.18* | 3.29 ± 0.18* | 19.86 ± 0.34* | 66.19 |
| III | 600 | 8 | 3.57 ± 0.30* | 1.86 ± 0.26* | 4.71 ± 0.36* | 3.14 ± 0.26* | 20.43 ± 0.57* | 68.09 |
| IV | 700 | 8 | 2.86 ± 0.34* | 1.43 ± 0.20* | 3.86 ± 0.26* | 2.57 ± 0.20* | 22.42 ± 0.48* | 74.73 |
| V | 800 | 8 | 2.00 ± 0.31* | 1.14 ± 0.14* | 3.43 ± 0.37* | 2.29 ± 0.18* | 23.29 ± 0.42* | 77.62 |

* = Significant P<0.05 compared to control.

$$\text{Diestrus index} = \frac{\text{Number of days with clear diestrus smear}}{\text{Total duration of treatment (Days)}} \times 100$$

Table 2. Effect of mancozeb on healthy follicles of the ovaries in albino rats

| Groups | Treatment (mg/kg/d) | No. of rats | Follicular size in μm (diameter) M ± S.E. | | | | | | Total number of healthy follicles |
|--------|---------------------|-------------|--|---------------|----------------|---------------|---------------|--------------|-----------------------------------|
| | | | I < 90 | II 91–260 | III 261–350 | IV 351–430 | V 431–490 | VI >491 | |
| I | Control + oil | 4 | 160.75 ± 1.45 | 63.75 ± 0.94 | 57.75 ± 0.47 | 19.00 ± 0.40 | 15.25 ± 0.47 | 3.25 ± 0.25 | 319.75 ± 2.05 |
| II | 500 | 4 | 156.50 ± 1.84 | 57.00 ± 0.70* | 53.00 ± 0.81* | 18.00 ± 0.40 | 15.00 ± 0.40 | 2.75 ± 0.25 | 301.75 ± 2.09* |
| III | 600 | 4 | 141.50 ± 1.44* | 46.00 ± 0.40* | 47.00 ± 0.70* | 16.00 ± 0.40* | 13.25 ± 0.47* | 1.50 ± 0.28* | 265.25 ± 2.35* |
| IV | 700 | 4 | 133.50 ± 1.55* | 39.25 ± 0.75* | 37.00 ± 0.70* | 11.00 ± 0.57* | 11.00 ± 0.40* | 1.25 ± 0.47* | 233.00 ± 1.73* |
| V | 800 | 4 | 94.00 ± 1.68* | 28.75 ± 0.47* | 29.00 ± 0.57* | 6.25 ± 0.94* | 10.00 ± 0.40* | 1.00 ± 0.40* | 168.75 ± 1.25* |

* = Significant P<0.05 compared to control.

Table 3. Effect of mancozeb on atretic follicles of the ovaries in albino rats

| Groups | Treatment (mg/kg/d) | No. of rats | Follicular size in μm (diameter) M ± S.E. | | | | | | Total number of atretic follicles |
|--------|---------------------|-------------|--|---------------|----------------|---------------|--------------|-------------|-----------------------------------|
| | | | I < 90 | II 91–260 | III 261–350 | IV 351–430 | V 431–490 | VI >491 | |
| I | Control + oil | 4 | 8.25 ± 0.25 | 13.75 ± 0.25 | 7.50 ± 0.28 | 6.25 ± 0.25 | 2.75 ± 0.30 | 1.75 ± 0.25 | 40.25 ± 0.07 |
| II | 500 | 4 | 9.25 ± 0.25 | 14.25 ± 0.47 | 7.25 ± 0.28 | 6.50 ± 0.28 | 2.75 ± 0.25 | 1.50 ± 0.50 | 41.75 ± 0.14 |
| III | 600 | 4 | 10.00 ± 0.40* | 13.25 ± 0.62 | 8.25 ± 0.25 | 7.25 ± 0.25* | 3.25 ± 0.25 | 1.75 ± 0.25 | 43.25 ± 0.85* |
| IV | 700 | 4 | 10.50 ± 0.50* | 15.75 ± 0.47* | 9.50 ± 0.28* | 7.75 ± 0.25* | 3.50 ± 0.28 | 2.00 ± 0.00 | 47.50 ± 1.04* |
| V | 800 | 4 | 12.00 ± 0.40* | 15.50 ± 0.28* | 9.25 ± 0.25* | 7.50 ± 0.28* | 3.25 ± 0.25 | 2.25 ± 0.25 | 49.75 ± 0.48* |

* = Significant P<0.05 compared to control.

Morphometric analysis of follicular growth studies

There is a significant decrease in the number of healthy follicles in stage II, III and total number of healthy follicles with 500 mg/kg/d mancozeb treatment. In higher doses of mancozeb treatment showed a significant decrease in the number of healthy follicles with concomitant increase in the number of atretic follicles dose-dependently (Tables 2, 3). The histological observations revealed normal number of developing follicles, Graafian follicles, Corpora lutea and atretic follicles in control rats (Fig. 1). The histologic examination of the ovary with 500 mg/kg/d mancozeb treated

rats revealed many developing follicles, Graafian follicles, Corpora lutea and atretic follicles (Fig. 2). The histologic observation of the ovary with 600, 700 and 800 mg/kg/d mancozeb treated rats revealed fewer developing follicles, less number of corpora lutea and many atretic follicles and the size of the ovary was also reduced when compared with control rats (Figs. 3–5).

Body and organs weight studies

The percent increase in the body weight of control rats is 5.05 g, when compared with that of the initial body weight.

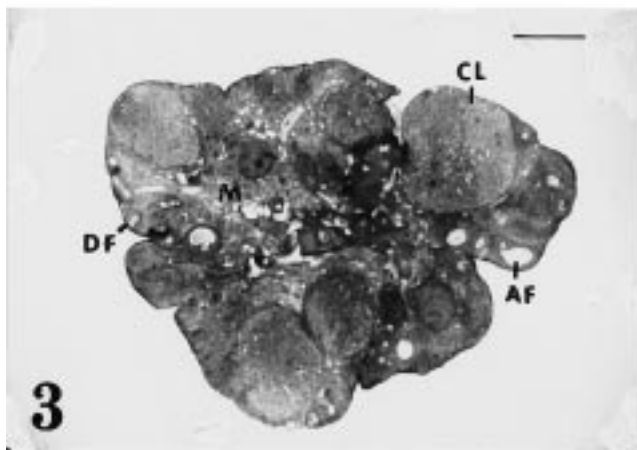
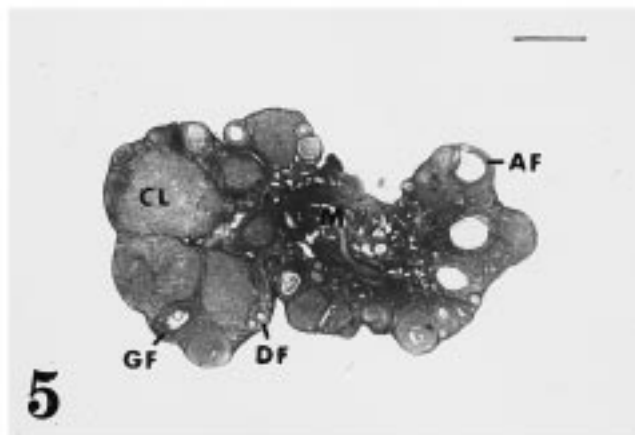
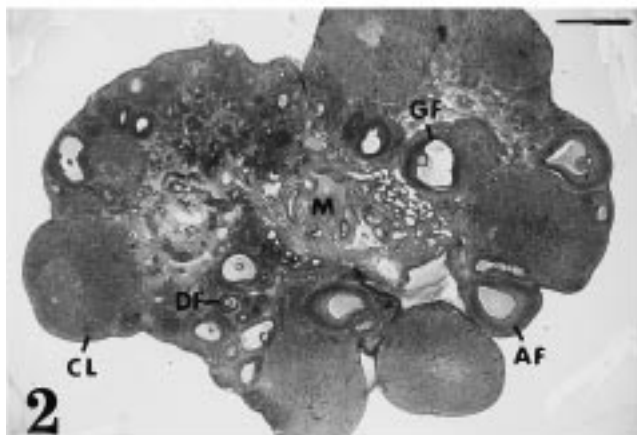
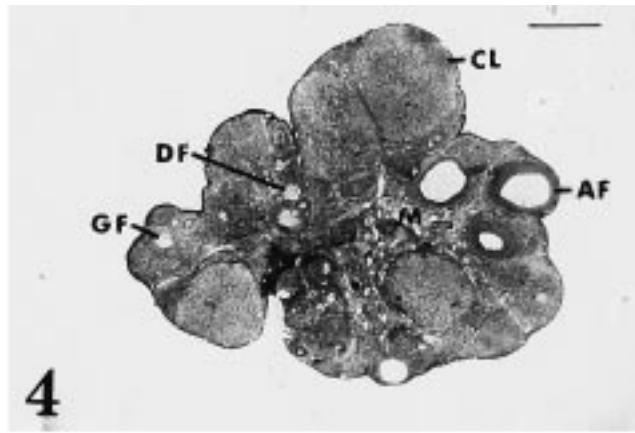
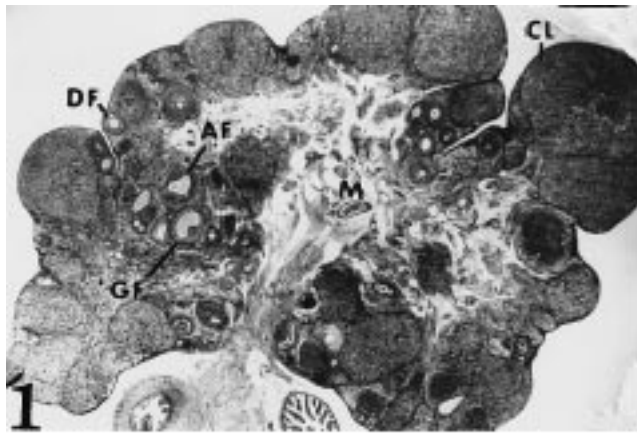


Fig. 1 Transverse section of the ovary from control rats showing many developing follicles(DF), Graafian follicle (AF), many corpora lutea (CL) and few atretic follicles(AF); Scale line= 500 μ m.

Fig. 2 Transverse Section of the ovary from rat treated with 500 mg mancozeb treated rat showing many developing follicles(DF), corpora lutea(CL) and atretic follicles(AF); Scale line = 500 μ m.

Fig. 3 Transverse section of the ovary with 600 mg mancozeb treated rat showing developing follicles(DF), corpora lutea(CL) and atretic follicles (AF); Scale line=500 μ m.

Fig. 4 Transverse section of the ovary with 700 mg mancozeb treated rat showing few developing follicles(DF), corpora lutea(CL) and many atretic follicles(AF); Scale line=500 μ m.

Fig. 5 Transverse section of the ovary with 800 mg mancozeb treated rat showing few developing follicles(DF), few small corpora lutea(CL) and many atretic follicles(AF); Scale line=500 μ m.

Table 4. Effect of mancozeb on body and organs weight in albino rats

| Groups | Treatment (mg/kg/d) | No. of rats | % Change in the | Relative weight (g / 100 g body weight M ± S.E.) | | | | | | | | | |
|--------|---------------------|-------------|-----------------|--|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|
| | | | | B.wt. | Ovary | Uterus | Kidneys | Adrenal | Spleen | Liver | Lungs | Heart | Thymus |
| I | Control + oil | 8 | 5.05 | 0.04±0.01 | 0.23±0.02 | 0.89±0.05 | 0.03±0.00 | 0.52±0.01 | 4.65±0.33 | 0.71±0.11 | 0.43±0.02 | 0.15±0.01 | 0.01±0.00 |
| II | 500 | 8 | -2.54 | 0.04±1.60 | 0.12±0.02 | 0.88±0.05 | 0.03±0.01 | 0.27±0.05 | 4.42±0.45 | 0.78±0.03 | 0.40±0.02 | 0.16±0.00 | 0.01±0.00 |
| III | 600 | 8 | -3.27 | 0.03±0.20 | 0.16±0.01 | 0.98±0.07 | 0.05±0.00 | 0.22±0.22 | 4.61±0.13 | 0.68±0.03 | 0.42±0.02 | 0.16±0.01 | 0.02±0.00* |
| IV | 700 | 8 | -4.63 | 0.04±1.91 | 0.16±0.02 | 0.94±0.03 | 0.04±0.00 | 0.47±0.01 | 5.05±0.48 | 0.77±0.01 | 0.41±0.02 | 0.15±0.01 | 0.04±0.00* |
| V | 800 | 8 | -6.76 | 0.04±0.00 | 0.13±0.01 | 1.06±0.04 | 0.05±0.00 | 0.17±0.01 | 5.55±0.22 | 0.84±0.05 | 0.51±0.02 | 0.15±0.00 | 0.08±0.00* |

* = Significant P<0.05 compared to control.

Table 5. Effect of mancozeb on biochemical constituents of the ovary in albino rats

| Groups | Treatment (mg/kg/d) | No. of rats | (Mg/g wet weight of tissue: M ± S.E.) | | | | |
|--------|---------------------|-------------|---------------------------------------|--------------|---------------|---------------|----------------|
| | | | Protein | Glycogen | Total lipids | Phospholipids | Neutral lipids |
| I | Control + oil | 4 | 130.66 ± 1.16 | 6.90 ± 0.17 | 85.00 ± 1.60 | 27.08 ± 0.22 | 51.50 ± 0.67 |
| II | 500 | 4 | 131.50 ± 0.50 | 6.50 ± 0.17 | 81.40 ± 1.74 | 27.83 ± 0.17 | 50.54 ± 0.46 |
| III | 600 | 4 | 130.50 ± 0.00 | 5.98 ± 0.12 | 83.56 ± 1.37 | 28.00 ± 0.14 | 51.33 ± 0.17 |
| IV | 700 | 4 | 123.33 ± 0.33* | 4.70 ± 0.09 | 86.91 ± 1.60 | 29.83 ± 0.44* | 53.08 ± 0.36* |
| V | 800 | 4 | 122.33 ± 0.33* | 3.50 ± 0.11* | 89.90 ± 1.80* | 30.08 ± 0.36* | 53.41 ± 0.30* |

*=Significant P<0.05 compared to control.

Table 6. Effect of mancozeb on biochemical constituents of the uterus in albino rats

| Groups | Treatment (mg/kg/d) | No. of rats | (Mg/g wet weight of tissue: M ± S.E.) | | | | |
|--------|---------------------|-------------|---------------------------------------|--------------|---------------|---------------|----------------|
| | | | Protein | Glycogen | Total lipids | Phospholipids | Neutral lipids |
| I | Control + oil | 4 | 111.60 ± 0.44 | 3.27 ± 0.07 | 62.08 ± 0.22 | 20.08 ± 0.11 | 31.16 ± 0.36 |
| II | 500 | 4 | 112.33 ± 0.66 | 3.05 ± 0.04 | 61.58 ± 0.30 | 20.25 ± 0.25 | 30.83 ± 0.22 |
| III | 600 | 4 | 112.16 ± 1.36 | 2.88 ± 0.06* | 58.91 ± 0.50* | 20.00 ± 0.25 | 30.08 ± 0.14 |
| IV | 700 | 4 | 109.33 ± 1.16 | 2.58 ± 0.15* | 58.50 ± 0.29* | 18.00 ± 0.58* | 29.16 ± 0.17* |
| V | 800 | 4 | 107.33 ± 0.66* | 1.99 ± 0.03* | 55.50 ± 0.76* | 17.83 ± 0.17* | 26.33 ± 0.89* |

*=Significant P<0.05 compared to control.

There is no significant decrease in the body weight of all the mancozeb treated rats when compared with control rats. The weight of the ovary, uterus, kidney, adrenal, spleen, liver, lungs, heart and thymus were not changed significantly in all the mancozeb treated rats when compared with those of the corresponding parameters of the control rats. However, the thyroid weight was significantly increased in all the mancozeb treated rats except 500 mg/kg/d when compared with control rats. Some animals were in estrus phase with mancozeb treatment at the time of autopsy (Table 4).

Biochemical studies

In ovary, there is a significant decrease in the levels of protein, glycogen and a significant increase in the levels of

total lipids, phospholipids with 700 and 800 mg/kg/d mancozeb treatment when compared with control rats (Table 5). In uterus, there is a significant decrease in the levels of protein, glycogen, total lipids, phospholipids and neutral lipids with 600, 700 and 800 mg/kg/d mancozeb treatment when compared with control rats (Table 6). In liver there is significant increase in the levels of total lipids and neutral lipids in all the mancozeb treatment when compared with control rats (Table 7).

Discussion

The control rats exhibited regular estrous cycle of 4–5 days. Cyclic changes of the vaginal smear observed in the

Table 7. Effect of mancozeb on biochemical constituents of the liver in albino rats

| Groups | Treatment (mg/kg/d) | No. of rats | (Mg/g wet weight of tissue: M ± S.E.) | | | | |
|--------|---------------------|-------------|---------------------------------------|--------------|---------------|---------------|----------------|
| | | | Protein | Glycogen | Total lipids | Phospholipids | Neutral lipids |
| I | Control + oil | 4 | 128.00 ± 0.50 | 15.89 ± 0.11 | 40.00 ± 0.43 | 9.75 ± 0.14 | 22.08 ± 0.36 |
| II | 500 | 4 | 126.66 ± 0.16 | 15.33 ± 0.11 | 42.00 ± 0.43* | 10.08 ± 0.22 | 22.66 ± 0.22 |
| III | 600 | 4 | 127.16 ± 0.44 | 15.09 ± 0.10 | 47.50 ± 0.29* | 9.85 ± 0.71 | 22.25 ± 0.38 |
| IV | 700 | 4 | 126.33 ± 0.33 | 14.76 ± 0.22 | 48.75 ± 0.14* | 11.58 ± 0.30* | 26.16 ± 0.93* |
| V | 800 | 4 | 126.17 ± 0.66 | 15.35 ± 0.14 | 50.41 ± 1.44* | 11.91 ± 0.46* | 27.41 ± 0.71* |

* = Significant P<0.05 compared to control.

estrous cycle gives a reasonable index of ovarian activity and its hormonal synthesis of estrogen and progesterone. The level of estrogen and progesterone are controlled by pituitary gonadotropins and hypothalamus-releasing gonadal hormone¹³. The data obtained in the present study reveals that the rats treated with mancozeb causes a significant decrease in the number of estrous cycle and duration of proestrus, estrus and metestrus with concomitant significant increase in diestrus phase. Diestrus index was also increased dose-dependently in all the groups following the administration of mancozeb treatment. It was dose dependent, similar results have been reported with other organophosphorus pesticides dimethoate, sumithion and methyl parathion in rats¹⁴⁻¹⁶. The alteration in the estrous cycle with prolonged diestrus in mancozeb treated rats may be due to the hormonal imbalance in the estrogen: progesterone ratio.

Greenwald¹⁷ has stated that intriguing mysteries in what factors determine, whether one follicle remains quiescent and another begins to develop but, other becomes atretic, while still a third matures and ovulates. Greenwald¹⁸ has also suggested that folliculogenesis has attracted a great deal of attention and can be applicable to reproductive toxicology. Peters and McNatty¹⁹ have reported that the ovary is an organ that is never at rest and follicles start to grow at all times and as they develop large number of cells. It has been reported that gonadotropic hormones are unessential for early growth of follicles and that FSH and LH become indispensable for further development only at the time of transformation of the secondary to a tertiary follicle²⁰. The classification of follicles in the mouse proposed by Pedersen and Peters²¹ provides an excellent framework for studying follicular kinetics in this study. Follicles start to grow at all times and as they develop, they produce more number of thecal and granulosa cells. The conversion of follicles to atretic state is functional rather than a degenerative process and it is considered to be an integral part of ovarian function²².

Hirshfield and Midgely⁸ have reported that atresia is a prominent feature in all vertebrate ovaries irrespective of age, stages of the reproductive phase and environmental factors²³. Most of the follicles undergo atresia, very few follicles mature and ovulate among the new crop of recruited follicles during every cycle. Osman⁹ has reported that in small pre-antral follicles the atresia affects the oocyte first followed by degeneration in granulosa cells. But in antral follicles the degenerative changes are seen in granulosa first. Subsequently, the oocyte gets affected resulting into processes such as resumption of meiosis and oocyte fragmentation as in rats. The method adopted by Kaur and Guraya²⁴ the follicles were classified on the basis of granulosa layers was not used in the recent study. Classifying the follicles on the basis of the number of granulosa cells in the largest section²¹ was not followed in the present study owing to the possibility of uneven distribution of granulosa cells in large follicles. Therefore, according to the method followed by Hirshfield and Midgely⁸ the follicular diameter was taken as the criteria to classify the follicles in the present study. In the serial sections, follicles containing oocyte and nucleolus were measured and counted, this method was found to be most convenient and appropriate.

The data obtained in the present study on follicular dynamics has revealed a significant decrease in the number of healthy follicles and increase in the number of atretic follicles in mancozeb treated rats. Similar findings have been reported on the reduction of different types of healthy follicular stages with concomitant increase in the atresia in rats and mice treated with different pesticides. Swartz and Mall²⁵ have reported that chlordecone induces follicular toxicity in reducing the pool of healthy, large and medium sized follicles with increase in the atretic follicles. It has been reported that the methoxychlor, dicofol and edifenphos treatment to adult rats increased the large regressing follicles and reduced the total number of healthy follicles²⁶⁻²⁸. Cyclophosphamide has been found to inhibit the development

of antral follicles in rats, thereby increasing the atretic follicles through interfering with hormonal ovarian follicular development and reduces estradiol²⁹). Evans *et al.*³⁰ have shown that the ovarian androgen and inhibin secretion by follicles may be an important part in the regulation of FSH secretion and follicular dynamics.

In the present study, there is also the possibility that the decrease in healthy follicles with concomitant increase in atretic follicles in rat may be due to affecting gonadotropin secretion via central nervous system mechanism, as it was observed in the rats with the following administration of dithiocarbamates³¹). Therefore, the reason may be due to the hormonal imbalance in any of the stages in hypothalamo-hypophysial ovarian axis or by insensitising the follicular receptors to the available gonadotropins thereby led to the retardation of further development of surviving follicles into next successive follicular stages and also arrest of estrogen production. But the exact nature of antigonadotropic mechanism of action in the present kind of follicular toxicity of mancozeb cannot be concluded from this study. Therefore, further investigations are still remained to be explored to know the mechanism of action of mancozeb on follicular development.

The administration of different dose treatment mancozeb showed decreased body weight in dose dependent manner, but was not significant. Although food intake has not been measured in this study, this may be one of the reason for low weight gain. This supports findings of Lu and Kennedy³²). Treatment with mancozeb in different doses did not affect the weights of ovary, uterus, kidney, adrenal, spleen, liver, lungs, heart and thymus. However, the thyroid weight was significantly increased in all the mancozeb treated rats except 500 mg treatment. The increase in the thyroid weight is also reported with zeneb aminotriazole and mancozeb treatment in animals³³⁻³⁶). It has been reported that increase in the weight of the thyroid gland of rat exposed to mancozeb may be due to ethylenethiouria (ETU) and carbondisulphid which are the major metabolites of mancozeb³⁷). It has been reported that the increase in the weight of thyroid gland of rat exposed to mancozeb found to be associated with hypertrophy and hyperplasia of the follicular cells causing structural and functional changes in the thyroid, as evidenced by inhibition of thyroid peroxidase, thyroid ratio iodine ¹²⁵I uptake, serum-protein-bound iodine (PB¹²⁵I), thyroid protein and thyroxine (T₄)³.

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 has revealed that the levels of protein in the ovary and uterus was significantly decreased. Recently similar results have been reported that the levels of protein in ovary and uterus decreases with mancozeb treatment in hemicastrated albino rats³⁸). The acute treatment with monocrotophos showed tissue specific inhibition of microsomal Cyt P-450 in hepatic and extra hepatic tissues resulted in the loss of haemoprotein in rats³⁹). Similar results were reported in rats treated with dimethoate⁴⁰). It has been reported that diethyl dithiocarbamate (DEDC) inhibits hepatic Cyt P-450 dependent enzyme activity in rats⁴¹). It has been suggested that there is a significant decrease in the levels of blood glucose and globulin in mancozeb treated rats, due to low thyroxine level because of impaired thyroid function⁴²).

The data obtained in the present study reveals a significant decrease in the levels of glycogen in ovary and uterus with mancozeb treatment. Manorajtham *et al.*⁴³ have reported that glycogen concentration and the activities of enzymes such as lactate dehydrogenase (LDH) isoenzymes and phosphomonoesterases were altered in testes with pesticide neem oil of plant origin in albino rats. The results suggests that the anabolic effects of zearalenone within the carbohydrate metabolism can be mediated by insulin⁴⁴). Recently, similar results has been reported that the glycogen level in ovary and liver was significantly decreased in hemicastrated rats treated with mancozeb³⁷).

The data obtained in the present study reveals that the levels of total lipids, phospholipids and neutral lipids were significantly increased in ovary and liver and decreased in uterus. It has been reported that increase in the level of phosphoinositides and phosphatidic acid in liver suggest the likely involvement of phospholipase C-pathway of signaling in the toxicity of mancozeb in different tissues varying levels⁴⁵).

It has been reported that mancozeb shows its biological effects through its metabolites like ethylene thiourea (ETU) and carbon disulphide (CS₂)^{46,47}). The changes in the levels of protein, glycogen and 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 function⁴⁷). Mancozeb, though having acute mammalian toxicity exhibited significant toxicological effects after repeated chronic exposure. This study has relevance since suggests a common judicious use of mancozeb for agriculture and occupational practice.

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