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

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Abstract: Mancozeb—a fungicide of ethylenebisdithiocarbamate group was orally administered at doses of 500, 1,000 and 1,500 mg/kg body weight/day for 30, 90, 180 and 360 days. Signs of toxicity mortality pattern and loss in body weight were observed in dose dependent manner. However, signs of intoxication and mortality pattern were more pronounced till the exposure of 90 days. A significant increase in testes and decrease in epididymis weight were associated with degeneration in seminiferous and epididymal tubules with loss of sperms. The decrease in gonadal acid phosphatase (ACP), succinic dehydrogenase (SDH) and increase in alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activity were observed with increased serum cholesterol. Sialic acid and protein content of testis and epididymis were also decreased in dose dependent manner. The study has thus indicated marked biochemical and pathological changes in gonads of male rats after chronic exposure to mancozeb.

Key words: Mancozeb, Testes, Epididymis, Male rats, Pathology, Enzymes

Introduction

Dithiocarbamates used in agriculture have been reported in a variety of ailments in workers1-3. Though women workers exposed to ethylenebisdithiocarbamates have shown toxicity during pregnancy4 and disorders in the menstrual cycle5, there is no information on male workers engaged in the same job. Mancozeb (Fig. 1), a fungicide of ethylenebisdithiocarbamate (EBDC’s) group is a polymeric complex of 20% manganese with 2–5% zinc salt. Since mancozeb is used against a variety of folliar fungal diseases and seed treatment, it has significance over other dithiocarbamates. Mancozeb is a grayish yellow powder and is stable under normal storage condition but decomposes at higher temperature by moisture and acid6. In general, EBDC’s have low mammalian toxicity but animal studies with mancozeb have shown its effects on skin, thyroid, central nervous system and on fetus. It has also been reported as tumor initiator and promoter on mouse skin2,3,7-9.

In view of paucity of information on reproductive system, the present study has been undertaken to know the possible long term effects of mancozeb on testes and epididymis of rats.

Materials and Methods

Two hundred male albino rats (Rattus norvigicus, Druckerey strain) of Industrial Toxicology Research Centre’s colony with an average body weight of 100 ± 10 g were
housed in an air conditioned (75 ± 2°F) room with relative humidity (55–70%) and 12:12 light and dark cycles. Animals were given synthetic pellet diet (Liptons India Ltd., India) and water ad libitum throughout the study. Animals acclimatized for 7 days prior to experimentation were divided into four groups each having 50 animals. Animals of the treatment groups were orally administered to mancozeb at doses 500, 1,000 and 1,500 mg/kg/day (6 days/wk) mixed with peanut oil over a period of 360 days. Control animals were given peanut oil in the similar fashion. Body weight was recorded daily and monitored at 360 days to find out the percent weight gain/loss of the rats.

During the treatment period on 30, 90, 180 and 360 days, six animals from each group were decapitated and blood was collected directly from jugular vein into non-oxalated tubes for the estimation of serum cholesterol. The testes and epididymis were removed quickly and weighed individually for their relative weight.

Histopathological studies
The testes and epididymis of each animal were fixed quickly in Bouin’s fluid. After routine processing, paraffin sections were cut at 5 µm and stained with haemotoxyline-eosine for microscopic examination.

Biochemical studies
Freshly removed testes and epididymis were washed free from extraneous material using chilled saline solution and homogenized in 0.25 M ice cold sucrose solution (10%, w/v) in a potter Elvehjem type homogenizer. The homogenate was centrifuged at 700 x g for 10 min and supernatant were used for estimation of enzymes. Activities of alkaline phosphatase (ALP, E.C. 3.1.3.1) and acid phosphatase (ACP, E.C. 3.1.3.2) were estimated by the method of Wootton. The activity of succinate dehydrogenase (SDH, E.C.1.3.99.1) was estimated by the method of Slater and Bonner, while the activity of lactate dehydrogenase (LDH, E.C. 1.1.1.27) was determined by the method of Bergmeyer and Bernt. The contents of protein and sialic acid were measured by the method of Lowry et al. and Aminoff. The level of cholesterol in serum was measured by the method of Zlatkis et al. Statistical significance between the control and experimental values was calculated according to the Student’s ‘t’-test.

Results
Mancozeb at all doses, i.e., 500, 1,000 and 1,500 mg/kg/d to male rats for 360 days produced signs of poisoning such as diarrhoea, dyspnea, salivation, nasal bleeding, hind limb paralysis in majority of animals and death of few animals. A dose dependent signs of toxicity appeared in the early stage of mancozeb administration. The mortality of animals during days 0–30, 30–90, 90–180 and 180–360 is shown in Table 1. The mortality was more pronounced during days 0–90 as compared to days 90–360. Although the body weight of control and treated animals have shown an increase, the percent increase was in order of 133>116>85>80 of rats exposed to mancozeb (0, 500, 1,000, 1,500 mg/kg/d), respectively (Table 1).

The absolute weight of testes and epididymis of animals exposed to different doses (500, 1,000 and 1,500 mg/kg/d) of mancozeb did not show any change except for a mild decrease (p<0.05) in epididymal weight at 1,000 and 1,500 mg/kg/d for 360 days. The relative weight of testes and epididymis after the exposure to different doses of mancozeb
(500, 1,000 and 1,500 mg/kg/d) during the period of 30–90 days did not indicate any appreciable change. However, higher doses (1,000 and 1,500 mg/kg/d) over a period of 180 and 360 days produced a slight increase in relative weight of testes and a decrease in epididymis (Table 2).

**Histopathological studies**

The testes and epididymis of control group of animals showed normal microscopic structure (Figs. 2, 3). Microscopic examination of testes and epididymis of rats exposed to mancozeb at doses 500 and 1,000 mg/kg/d over a period of 360 days did not exhibited any significant pathomorphological changes. However, testes of rats dosed with mancozeb (1,500 mg/kg/d) produced time dependent pathological changes in seminiferous tubules. The observed changes included necrotic seminiferous tubules with sloughing of germinal cells, formation of giant cells and accumulation of debris matter into the lumen. The clubing of spermatogonia and spermatocytes may be responsible for the formation of giant cells. Similarly rats exposed to high doses of mancozeb has also showed damaged epithelial cells in the tubules of epididymis with loss of sperms (Figs. 4–6).

**Biochemical studies**

The lower dose (500 mg/kg/d) of mancozeb did not show any change in the activities of testicular and epididymal ACP, ALP, LDH and SDH during 90 days. However, a marginal to significant (p<0.05–0.001) alteration of these enzymes was observed during 90–360 days. In contrast, higher doses (1,000 and 1,500 mg/kg/d) of mancozeb has significantly reduced (p<0.05–0.001) the activity of ACP and SDH in

<table>
<thead>
<tr>
<th>Days</th>
<th>Treatment (mg/kg/d)</th>
<th>Absolute weight (g)</th>
<th>Relative weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testes</td>
<td>Epididymis</td>
<td>Testes</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>2.13 ± 0.17</td>
<td>0.85 ± 0.059</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1.90 ± 0.19</td>
<td>0.76 ± 0.048</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>2.11 ± 0.25</td>
<td>0.62 ± 0.088</td>
</tr>
<tr>
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<td>1,500</td>
<td>2.06 ± 0.26</td>
<td>0.57 ± 0.099</td>
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<tr>
<td>360</td>
<td>0</td>
<td>2.70 ± 0.25</td>
<td>0.90 ± 0.096</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2.19 ± 0.25</td>
<td>0.81 ± 0.034</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>2.43 ± 0.15</td>
<td>0.58 ± 0.088</td>
</tr>
<tr>
<td></td>
<td>1,500</td>
<td>2.60 ± 0.21</td>
<td>0.54 ± 0.094</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE of six rats. * p<0.001, ** p<0.01, *·* p<0.02, ··· p<0.05.

**Fig. 2.** Section of testes of control rats showing normal structure of seminiferous tubules
H & E × 375.

**Fig. 3.** Section of epididymis of control rats showing normal structure of tubules filled with mature sperms
H & E × 156.

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both testes and epididymis during 360 days. On the other hand, the activity of LDH and ALP was found to be significantly increased (p<0.05–0.001) at all the intervals of animals exposed to mancozeb (1,000 and 1,500 mg/kg/d). The level of serum cholesterol showed a dose and time dependent increase (p<0.05–0.001) at all the treatment groups of rats exposed to mancozeb (Figs. 7–9). The content of protein and sialic acid in testes and epididymis was found to be reduced (p<0.05–0.001) at higher dose (1,000 and 1,500 mg/kg/d) levels during 90–360 days exposure (Table 3).

Discussion

Three hundred and sixty days oral feeding of mancozeb to rats have produced severe signs of toxicity and deaths during initial stage (0–90 days) of feeding. The degree of intoxication and mortality was reduced in later stages (90–360 days). Short and long term exposures to several EBDC’s such as maneb, zineb and mancozeb have shown similar pattern of toxicity and death of animals. In contrast, Raizada et al. have reported lack of intoxication and mortality of rats after 30 days exposure to zineb (1,000 mg/kg/d). The reduced mortality rate in the later stages of exposure may be due to development of tolerance in animals against the mancozeb. Probably the death in animals is attributed to intoxication and general weakness.

It is interesting to note that a dose dependent loss in body weight was observed in treated animals as compared to control. Similarly, a significant depression in body weight gain in male rats exposed to mancozeb was also observed. Ivanova-Chemishanska has reported dose linked anorexia and general weakness in animals exposed to maneb, zineb and mancozeb. Probably this may be one of the reasons for weight loss in animals exposed to mancozeb in the present study (Food intake was not monitored).

A mild increase in the relative weight of the testes in the present study is in conformity to the findings of Szepvolgyi et al. and Hess et al. The subchronic toxicity study of dithane M45 (253 and 379 mg/kg/d) has also shown an increase in the testicular weight. Hess et al. reported that sloughing of germ cells has blocked the testicular
excurrent duct, which resulted in the accumulation of fluid and lead to an increase in testicular weight in animals exposed to benomyl. The testicular weight in animals exposed to ziram and thiram has also been found to be increased in earlier studies reported from this laboratory. In the present study sloughing of germinal cells and accumulation of debris matter along with formation of giant cells may be responsible for the weight increase of the testes.

Similarly, in the present study exposure of rats to mancozeb has been found to produce significant pathological changes in the testes and epididymis. Zineb as high as 1,000 mg/kg/d produced similar testicular damage in rats. Oral administration of maneb and zineb to rats has also caused testicular atrophy with damaged germinal epithelium and reduced sperm mortality and viability.

Mancozeb has been found to produce mortality, body weight loss, an increase in relative testicular weight and pathological changes. These changes were found to be corroborated with increased activity of LDH, ALP and decreased activity of ACP and SDH in testes and epididymis. This indicates the possible subtle disturbances taking place in the testicular function of the animals. It may be relevant to note that analysis of testicular enzymes act as an important tool for the assessment of testicular growth and development in animals. The present study has shown increased level of serum cholesterol. The enhanced level of serum cholesterol in the present study appears to influence the steroidogenic process of Leydig cells indicating the testicular dysfunction. Involvement of cholesterol in the
Fig. 8. The activity of ACP, ALP, SDH and LDH in epididymis of (a, b, c, d) rats treated with mancozeb. •= Control, o——o 500 mg/kg/d, △——△ 1,000 mg/kg/d, □——□ 1,500 mg/kg/d.

Table 3. Levels of sialic acid and protein in male rats after oral administration of mancozeb for 360 days

<table>
<thead>
<tr>
<th>Days</th>
<th>Treatment (mg/kg/d)</th>
<th>Sialic Acid (mg/g tissue)</th>
<th>Protein (mg/g tissue)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Testes</td>
<td>Epididymis</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>5.98 ± 0.125</td>
<td>5.13 ± 0.139</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5.73 ± 0.141</td>
<td>5.27 ± 0.145</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>5.50 ± 0.124^a</td>
<td>4.69 ± 0.153</td>
</tr>
<tr>
<td></td>
<td>1,500</td>
<td>5.21 ± 0.118^a</td>
<td>4.36 ± 0.118^a</td>
</tr>
<tr>
<td>360</td>
<td>0</td>
<td>6.00 ± 0.112</td>
<td>4.91 ± 0.146</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5.43 ± 0.129^c</td>
<td>4.75 ± 0.182</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>5.17 ± 0.134^c</td>
<td>4.40 ± 0.111^c</td>
</tr>
<tr>
<td></td>
<td>1,500</td>
<td>4.97 ± 0.165^c</td>
<td>4.18 ± 0.106^c</td>
</tr>
</tbody>
</table>

Value represents the mean ± SE of six animals. ^: p<0.001, ^: p<0.01, ^: p<0.02, ^: p<0.05.
testosterone synthesis and regulation of spermatogenesis is well known phenomenon\textsuperscript{28}. The changes in the level of protein and sialic acid caused maturational defects since the epididymal milieu is affected\textsuperscript{29} in hormone-injected rats thereby hampering fertilizing capacity. Decreased content of sialic acid and protein in the present study are correlated with morphological and enzymatic changes. Moderate pathological changes and alteration in enzyme profile of epididymis were observed in mancozeb-exposed rats. This is in confirmation with the reported changes in epididymis of rats exposed to thiiram\textsuperscript{30, 31}. The alteration in gonadal (testes/epididymis) enzyme activity along with increased testicular weight and significant pathological changes are suggested of gonadal damage. Therefore, the present study has indicated that chronic exposure to mancozeb induced biochemical and structural changes in gonads of male rats.

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References

14) Aminoff D (1961) Methods for the quantitative estimation of N-acetylneuraminic acid and their

Fig. 9. The levels of serum cholesterol in male rats treated with mancozeb


