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# MANCOZEB FUNGICIDE INDUCED TOXICITY IN LEVEL OF LIPID PER OXIDATION LPO AND HYDROGEN PEROXIDE $H_2O_2$ IN THE CRUDE HOMOGENATE, MITOCHONDRIAL AND MICROSOMEL RICH FRACTION OF TESTES IN ADULT ALBINO RATS

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**ABSTRACT** Mancozeb, a fungicide of ethylenebisdithiocarbamate (EBDC's) group is a polymeric complex of 20% manganese with 2-5% zinc salt. It is commonly used for foliar application and seed treatment in agriculture. Low acute toxicity, Mancozeb has been shown to produce adverse effects on reproduction, liver, kidney, central nervous system and chromosomes of bone marrow cells in mice and rats. The present study is undertaken to assess the protective effect of Vitamin C against Mancozeb induced toxicity in the Testis of adult albino rats. 90 days old adult male wistar rats (Rattus norvegicus) were exposed to Mancozeb at the dose of 300mg/kg body weight, orally for 60 days. The Control group received the Olive oil alone as vehicle. Administration of Mancozeb the levels of lipid peroxidation and hydrogen peroxide were significantly elevated in crude homogenate, Mitochondrial and Microsomal of testis in Mancozeb treated rats, when compared to control. The lipid peroxidation level and hydrogen peroxide remained normal in animals treated with vitamin C and Mancozeb. However, vitamin C treated rats did not show any significant alteration in the levels of lipid peroxidation and hydrogen peroxide. Further, withdrawal of Mancozeb restored the levels of $H_2O_2$ and LPO to normalcy, compared to control rats. The present study reveals the protective effect of vitamin C on the Mancozeb induced Testicular toxicity in the adult rats.

**KEYWORDS :** Mancozeb; VitaminC; Testes: Lipid peroxidation (LPO) Hydrogen peroxide($H_2O_2$)

## INTRODUCTION

Pesticides especially fungicides are widely and currently used in agriculture for eradication of fungal infection of grains, fruits, vegetables, flowers and tobacco crops from decay. In addition to their fungicidal applications, they are also used as seed disinfectants and insecticides. **Dithiocarbamates** are used to prevent crop damage in the field and to protect harvested crops from deterioration in storage or transport. Commercial products of dithiocarabamates fungicides include nabam, ziram, thiram, maneb, zineb and **Mancozeb**. The structure of these compounds is the same, except for the metal cation to which they are complexed (Casarett et al., 1996).

Mancozeb, a fungicide of ethylenebisdithiocarbamate (EBDC's) group is a polymeric complex of 20% manganese with 2-5% zinc salt. It is commonly used for foliar application and seed treatment in agriculture. Low acute toxicity, Mancozeb has been shown to produce adverse effects on reproduction, liver, kidney, central nervous system and chromosomes of bone marrow cells in mice and rats. (Sittig and Mane, 1991; Ksheerasagar and Kaliwal, 2003; Elzawahry, 2004; Joshi et al., 2005;).

Evidence are available to suggest that the Mancozeb has deleterious effect on various aspects of Male reproduction. However, the information on the toxic effect of this fungicide on male reproductive sex organs mainly testicular is restricted. Lipid peroxidation is now considered to be a major mechanism by which oxygen free radicals can cause tissue damage leading to impaired cellular function, alterations in the physico-chemical properties of cellular membranes, apoptosis and reduced enzyme/protein activity (Uchida, 2003).The present examination was undertaken to elucidate the effect of mancozeb on the level of Testicular Lipid pro-oxidation (LPO) and hydrogen peroxidation ($H_2O_2$) in the crude homogenate, Mitochondrial and Microsomel rich fraction of testes in adult albino rats.

## MATERIALS AND METHODS

### Chemicals

Mancozeb was a gift from the Agriculture Department, Government of Puducherry. Mancozeb (commercial grade 75% wettable powder) was made available from Indofil chemicals company, Mumbai. All other chemicals were of analytical grade and were purchased from locally through commercial sources.

### Animals

Healthy Male adult albino rats of Wistar strain weighing 200-210 g were housed in a clean polypropylene cages and maintained in the air conditioned animal house with constant 12 h/12h dark and light cycle .The animals were purchased from the Tamil Nadu veterinary and Animal Sciences University, Chennai. The animals were maintained and handled as per the guidelines given by the committee for the purpose of control and supervision of experimental on animals (CPCSEA), Government of India and Animal Ethical Committee(UAEC). The animals were fed with Standard rat pellet diet and clean drinking water was made available ad libitum.

## EXPERIMENTAL DESIGN

Adult male albino rats were divided into three groups and each group consists of six animals.

**Group I – Control:** Rats were given olive oil as vehicle orally, daily for 60 days.

**Group II – Mancozeb treatment**: Rats were treated with Mancozeb dissolved in olive oil at a dose of 300 mg/kg body weight ($1/10^{th}$ of $LD_{50}$) daily for 60 days, orally.

**Group III –Mancozeb with Vitamin C treatment**: Rats were treated with Mancozeb at a dose of 300 mg/kg body weight daily, orally along with Vitamin C (40 mg/kg body weight) for 60 days.

**Group IV –Withdrawal of Mancozeb treatment**: Rats were treated with Mancozeb at a dose of 300 mg/kg body weight in olive oil orally, daily for 60 days and withdrawal of the treatment for further period of 60 days.

## COLLECTION OF TISSUES

The rats were weighed and sacrificed twenty-four hours after the last treatment, by anaesthetic ether. The sex organ testes was removed cleaned of fat and adhering tissue, washed in cold physiological saline repeatedly weighed and kept on ice at 4ºC for further analysis.

## BIOCHEMICAL ANALYSIS

The testis were sonicated in ice-cold 0.1 M Tris-Hcl buffer (pH 7.4) and centrifuged at 800xg for 30 min at 4°C and the supernatant was collected and used for the biochemical analysis.

Mitochondrial and microsomal fractions of the testis were obtained by the differential centrifugation method as described by Chainy et al.(1997) after standardization. Briefly, a 20%(w/v) homogenate was prepared in ice-cold 0.25 M sucrose solution with the help of a motor-driven glass Teflon homogenizer. The homogenate was centrifuged at 1000 x g for 10 min at 4 °C to obtain the nuclear pellet. Mitochondrial pellet was obtained by centrifuging the post-nuclear supernatant at 10000 X g for 10 min at 4 °C . The microsomal pellet was prepared by the calciumchloride ($CaCl_2$) Sedimentation method of Kamath and Narayan (1972). Briefly, the post-mitochondrial supernatant was diluted with ice-cold $CaCl_2$ (1 M) so that the final concentration of $CaCl_2$ was 0.8 M. It was incubated at 4 °C for 10 min with occasional stirring. Then the sample was centrifuged at 10000 X g for 10 min at 4 °C and the microsomal pellet was obtained. All the fractions were washed thrice with ice-cold 1.15% potassium chloride solution and dissolved in the 0.25 M sucrose solution (1 mg protein/0.1 ml). The mitochondrial and microsomal fractions were used for biochemical studies.

Testicular tissue **lipid peroxidation** was measured by the method of Devasagayarn and Tarachand (1987).

**Hydrogen peroxide** production was assessed by the spectrophoto metric method of Holland and Storey (1981).

**STATISTICALANALYSIS:**

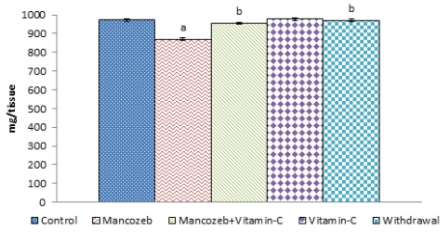
Single way Analysis of Variance (ANOVA) was followed to analyse the data according to **Zar** (1974). If the 'F'-ratio was significant, Student-Neumann-Keul's (SNK) test was followed.

**RESULT AND DISSCUSSION:**

The testicular weight was found to be decreased significantly in the Mancozeb treated rats (Figure.1). Co-administration of Vitamin C with Mancozeb registered normal testicular weight. Further, Vitamin C alone had no significant effect in the weight of the testis. Mancozeb withdrawn recovered the normal testicular weight. The reduction in the testicular weight observed in Mancozeb treated rats suggests the degenerating capacity of Mancozeb. Reduction in the weight of the testis could be due to inhibition of seminiferous tubule fluid formation and loss of germ cell by direct inhibition on spermatogenesis. Reduction in testicular weight is in agreement with earlier studies on rats (Joshi et al., 2005) and Swiss mice (Ksheerasagar et al., 2003).

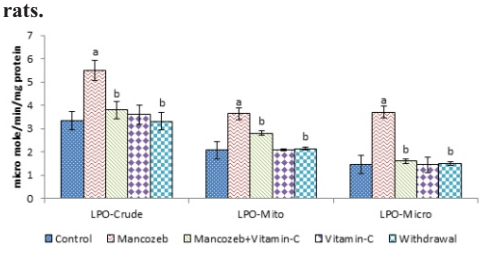
During normal steroidogenesis, LPO and ROS are produced by electron leakage outside the electron transfer chains and these oxygen radicals can initiate lipid peroxidation, to inactivate P450 enzymes (Hanukoglu et al., 1993). Several lines of evidence indicate that interactions between the testicular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

**Figure: 1. Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Testicular Weight in adult Male rats.**




**Each value is Mean± SEM of 6 Animals.**
**[a] and [b] represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.**

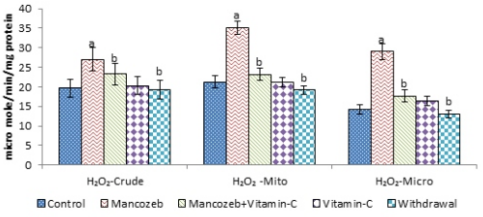
**Figure 2: Effect of Mancozeb treatment, Co-administration of Vitamin C with Mancozeb,Vitamin C alone and Withdrawal treatment on the level of LPO in Crude homogenate, Mitochondrial and Microsomal- rich fractions of Testis in adult Male rats.**




**Each value is Mean± SEM of 6 Animals.**
**[a] and [b] represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.**

In the present study the levels of lipid peroxidation (LPO) and reactive oxygen species such as hydrogen peroxide ($H_2O_2$) in the crude homogenate, Mitochondrial and Microsomal fractions of testis have been shown in Figure (2-3). The levels of lipid peroxidation and hydrogen peroxide were significantly elevated in crude homogenate, Mitochondrial and Microsomal of testis in Mancozeb treated rats, when compared to control. The lipid peroxidation level and hydrogen peroxide remained normal in animals treated with vitamin C and Mancozeb. However, vitamin C treated rats did not show any significant alteration in the levels of lipid peroxidation and hydrogen peroxide. Further, withdrawal of Mancozeb restored the levels of $H_2O_2$ and LPO to normalcy, compared to control rats

**Figure 3: Effect of Mancozeb treatment, Co-administration of Vitamin C with Mancozeb, Vitamin C alone and Withdrawal treatment on the level of $H_2O_2$ in Crude .homogenate, Mitochondrial and Microsomal-rich fractions of Testis in adult Male rats.**




**Each value is Mean± SEM of 6 Animals.**
**a and b represent statistical significant at P<0.05 Compared wit Control and Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.**

An elevation in LPO caused by other chemicals in different experiment as also been reported; methoxychlor(Latchoumycandane and Mathur,2002b), endosulfan (Kwon etal.,2005) and 2,3,7,8-tetrachlorobenzo-p-dioxin(TCDD) (Latchoumycandane et al., 2003). The decrease in activities of enzymatic antioxidants in the crude homogenate, mitochondrial and microsome rich fractions of testis exposed to Mancozeb might have increased LPO and ROS production. In the present study administration of Mancozeb, the levels of hydrogen peroxide($H_2O_2$) and lipid peroxidation(LPO) increased in the mitochondrial and microsomal fractions of the testis,

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