



## The Protective Effect of Vitamin-C against Mancozeb fungicide induced toxicity in Serum Hormone level in adult albino Rats

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### ABSTRACT

Mancozeb, an Inorganic-zinc dithiocarbamate is a typical fungicide with carbamate structure. It is chemically identified as ethylene-bis-dithiocarbamate (EBDC). It is commonly used for foliar application and seed treatment in agriculture. The present work was conducted to assess the protective effect of vitamin C against Mancozeb induced toxicity in the level of serum hormone in adult albino rats. 90 days old adult Male wister albino rats (*Rattus norvegicus*) were exposed to Mancozeb at the dose of 300mg/kg body weight, orally for 60 days. The control group received olive oil. Mancozeb treated significantly decreased the level of serum gonadotrophins (FSH and LH) and prolactin (PRL) and the level of serum Testosterone (T) and Estradiol (E) were found in serum of Male Rat. Co-administration of vitamin-C with Mancozeb and Vitamin-C alone restored the level of the serum hormone to normalcy. The withdrawal group of Mancozeb toxic effect was reversible. The present study reveals that the protective effect of Vitamin – C on the Mancozeb induced the level of serum hormone toxicity in albino adult rats.

### KEYWORDS

Mancozeb ;Vitamin C; FSH ,LH ,PRL;TestosteroneEstradiol; Serum Hormone.

### INTRODUCTION

Mancozeb a fungicide of ethylenebisdithiocarbamate (EBDC)'s group is a polymeric complex of 20% manganese with 2.5% zinc salt. Since mancozeb is used against variety of foliar fungal diseases and seed treatment. It is greyish yellow powder, normal storage condition but decomposes at higher temperature by moisture and acid (Worthing CR, 1991).

Mancozeb causes significant pathological changes were observed in Liver, Kidney,, Heart and also slight enlargement and brain revealed few petechial haemorrhages (Hore et.al., 1997). Male and Female reproductive system in rats (Baligar and Kaliwal 2001). Mancozeb has been shown to produce significant toxicological effect on thyroid gland in rat (kackar et al., 1997).

Evidence are available to suggest that the Mancozeb has deleterious effect on various aspects of adult albino rats. However, the information on the toxic effect of this fungicide on male albino rat mainly the level serum hormone is reduced. The present examination was undertaken to elucidate the effect of mancozeb on the serum hormone level (FSH,LH,PRL Testosterone Estradiol) of adult albino rats.

### Materials and Methods

#### Animals

Healthy Male adult albino rats of Wistar strain weighing 200-210 gm 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 (IAEC). The animals were fed with Standard rat pellet diet and clean drinking water was made available ad libitum.

#### Chemicals

Mancozeb (85% purity) was a gift from the Krishi Rasayn Exports Pvt.Ltd. Hyderabad, India. All other chemicals used were of analytical grade and were purchased locally through commercial sources.

#### Experimental Design

Adult male albino rats were divided into five 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 ( $1/10^{\text{th}}$  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- **Vitamin C alone treatment :** Rats were treated with Vitamin C alone, orally at a dose of 40 mg/kg body weight daily for 60 days.

Group V- **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.

#### Sample collection Procedures

The rats were weighed and sacrificed twenty-four hours after the last treatment, by anaesthetic ether and Trunk blood was collected in a clean dry test tube and then allowed to stand at room temperature for 10-15 min. The serum collected was then gently a spirited by spinning at 1,500 x g for 15 min at 4°C and used for hormone assays. All hormones were quantified by radioimmunoassay (RIA) using standard procedures, which have been standardized and used in the laboratory (see Bolton and Hunter, 1986). Testosterone and Estradiol were assayed by liquid phase RIA adopting the WHO procedure (Sufi et al., 1986).

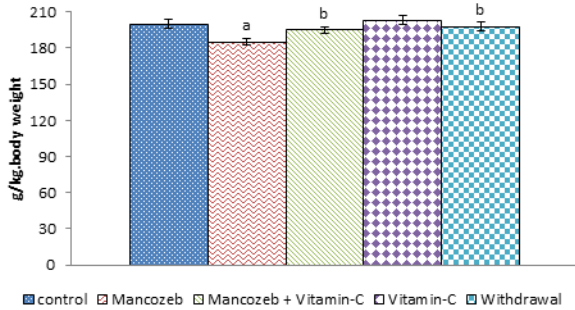
#### Statistical Analysis:

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.

#### Results and Discussion

Ethylene-bis-dithiocarbamate fungicide is toxic to the reproductive and endocrine system in different species ( Corisini,et al., 2005; Mills et al., 2005). Body weight display of protein and fat metabolism. The effect of Mancozeb at the dose of 300mg/kg body weight for 60 days on the body weight in adult albino rats has been presented in Figs (1). Administration of Mancozeb significantly decreased ( $p < 0.05$ ) the body weight compared to control rats. Co-administration of vitamin-C and Vitamin-C alone registered normal body weight compared to Mancozeb treated rats. However, the withdrawal of Mancozeb, restored the normal body weight. The reduction in body weight may be due to high rate of protein breakdown, which might be needed to fulfil energy requirements during detoxification (Ananthan.G and Kumaran.B. 2013). The another reason for weight loss in animals exposed to Mancozeb in the present study.

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



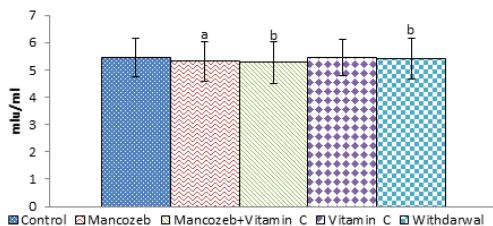
Each value is Mean± SEM of 6 Animals. <sup>a</sup> and <sup>b</sup> represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.

The level of serum gonadotrophins (FSH and LH) and prolactin (PRL) in the male rats treated with Mancozeb are presented in Figure (2-4). Neither the gonadotrophins nor PRL titres were affected by Mancozeb treatment. Similarly other treatment groups registered no appreciable alteration in the serum gonadotrophins and prolactin.

The levels of serum Testosterone (T) and Estradiol (E) were found to be significantly decreased (p<0.05) in Mancozeb treated rats. There was no significant change in serum levels of testosterone and estradiol in treatment of Co-administration of Mancozeb with Vitamin-C and Vitamin-C alone. The withdrawal of Mancozeb treatment, indicating the reversal of Mancozeb effects. Figure-(5and6)

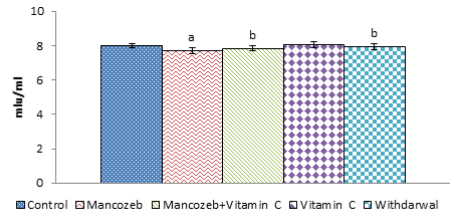
These results go hand in hand with the earlier investigation of Joshi et al., (2005). They reported that the decreased serum testosterone level observed in the Mancozeb treated rats at the dose of 500 mg/kg body weight for 30 days, orally, indicated the ability of Mancozeb to impair testosterone synthesis. Since, testosterone synthesis involves the active role of cytochrome P450 containing enzymes, their inhibition by Mancozeb might have resulted in altered circulating testosterone levels. Inhibition of implantation by Mancozeb due to hormonal imbalance or its toxic effects have been studied (Bindali and Kaliwal, 2002).

**Figure 2: Effect of Mancozeb treatment, Co-administration of Vitamin C with Mancozeb, Vitamin C alone and Withdrawal treatment on serum FSH levels in adult Male rats**



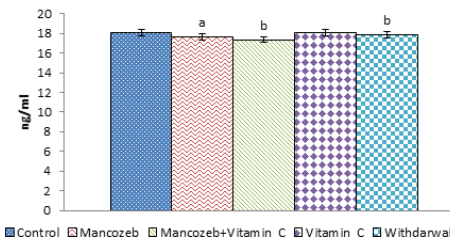
Each value is Mean± SEM of 6 Animals. <sup>a</sup> and <sup>b</sup> represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.

**Figure 3 : Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on serum LH levels in adult Male rats**



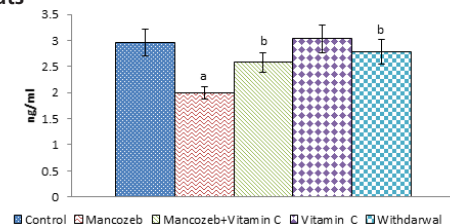
Each value is Mean± SEM of 6 Animals. <sup>a</sup> and <sup>b</sup> represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.

**Figure 4: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on serum Prolactin levels in adult Male rats**



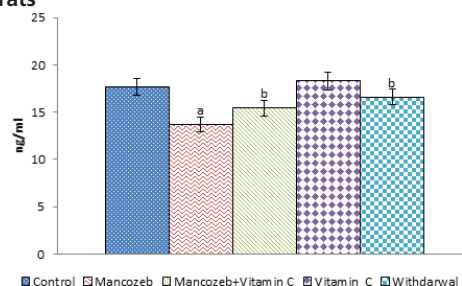
Each value is Mean± SEM of 6 Animals. <sup>a</sup> and <sup>b</sup> represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.

**Figure 5: Effect of Mancozeb treatment, Co-administration of Vitamin C with Mancozeb, Vitamin C alone and Withdrawal treatment on serum Testosterone levels in adult Male rats**



Each value is Mean± SEM of 6 Animals. <sup>a</sup> and <sup>b</sup> 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 6: Effect of Mancozeb treatment, Co-administration of Vitamin C with Mancozeb, Vitamin C alone and Withdrawal treatment on serum Estradiol levels in adult Male rats**



Each value is Mean± SEM of 6 Animals. <sup>a</sup> and <sup>b</sup> 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.

### **Mancozeb, respectively. Control Vs other groups; Mancozeb Vs Mancozeb + Vitamin C; Mancozeb Vs Withdrawal.**

In the present work the reduction in serum testosterone in Mancozeb treated rats may be a cumulative effect of decreased number of Leydig cells, testosterone synthesis and secretion. Probably, the decreased level of testosterone in serum could also be due to the diminished responsiveness of Leydig cells to LH and/or the direct inhibition of testicular steroidogenesis (Rajeswary et al., 2007a).

It has been shown that exposure to environmental contaminants adversely affects testicular function by decreasing pituitary LH secretion and reducing Leydig cell steroidogenesis (Murugesan et al., 2007). Together with gonadotrophins, testosterone is key hormones that regulate spermatogenesis. The secretion of testosterone by the Leydig cells is dependent upon the secretion of LH by the pituitary gland. Various pesticides and fungicides have been studied for their effect on testosterone level.

Therefore, the present study reveals that the treatment of Mancozeb is capable of inducing adverse effects on the serum hormone level in adult rats. However, Co-administration of Vitamin C with Mancozeb imparts protective effect on serum hormone level of adult rats. Vitamin C is an antioxidant found in both animals and plants (Smirnoff. 2001). Additionally, vitamin C is an tremendous essential scavenger (Sapper, et al., 1982; Upasani and Balaram, 2001) The restoration of serum level of hormone in the present study also indicates the protective effect of Vitamin C. This effect may be due to the antioxidant property and antimutagenic activity of Vitamin-C. Its antimutagenic property might be due to its antagonistic action at all probable level of pesticide genotoxicity (Goncharova, 1984). It is note that all parameters that were affected by Mancozeb treatment. However, The clearly indicates that these effects are transient and reversible withdrawal of Mancozeb treatment.

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