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Effect of Mancozeb on the Specific activities of Testicular Steroidogenic Enzymes (3β-HSD, 17β-HSD) and Protective role of Vitamin C in Albino Rats

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Abstract: ***Mancozeb, an Inorganic-zinc dithiocarbamate is a typical fungicide with carbamate structure. It is chemically identified as ethylenebisdithiocarbamate (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 Testicular activity of 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. Administration of Mancozeb significantly decreased (p<0.05) the activities of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β- hydroxysteroid dehydrogenase (17β-HSD), compared to control. Co-administration of vitamin-C with Mancozeb restored the activities of the enzymes 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 testicular toxicity in adult rats.***

Keywords: Mancozeb ;Vitamin C; 3β- and 17β - Hydroxysteroid dehydrogen ase (3β-HSD, 17β-HSD),Testes.

1. Introduction

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. It is also used to protect many fruits, vegetables, nuts and field crops against a wide spectrum of fungal diseases, including potato blight, leaf spot, scab and rust. Besides its fungicidal property, it is used in the industry as a slimicide in water cooling systems, a vulcanization accelerator, an antioxidant in the rubber industry, and as a metal scavenger in waste water treatment (Worthing, 1991). Despite its 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; Raghavendra et al., 2003; 2010; Elzawahry, 2004; Joshi et al., 2005; Salem, 2011).

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. The present examination was undertaken to elucidate the effect of mancozeb on the activity of Testicular Steroidogenic Enzymes 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β- hydroxysteroid dehydrogenase (17β-HSD), of adult albino rats.

2. Materials and Methods

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 4°C for further analysis.

Biochemical Analysis

The Tissue was homogenised in Teflon homogeniser (Potter Elvehjem) in Normal Saline and the homogenate was centrifuged at 10000 × g for 30 min at 4^{o} C .The Supernatant was used for various biochemical assays. Protein was determined according to the method of **Lowry, *et al*., (1951)**. The activity of 3β-HSD and 17β-HSD in Testicular tissue was determined by the method of Bergmeyer (1974).

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.

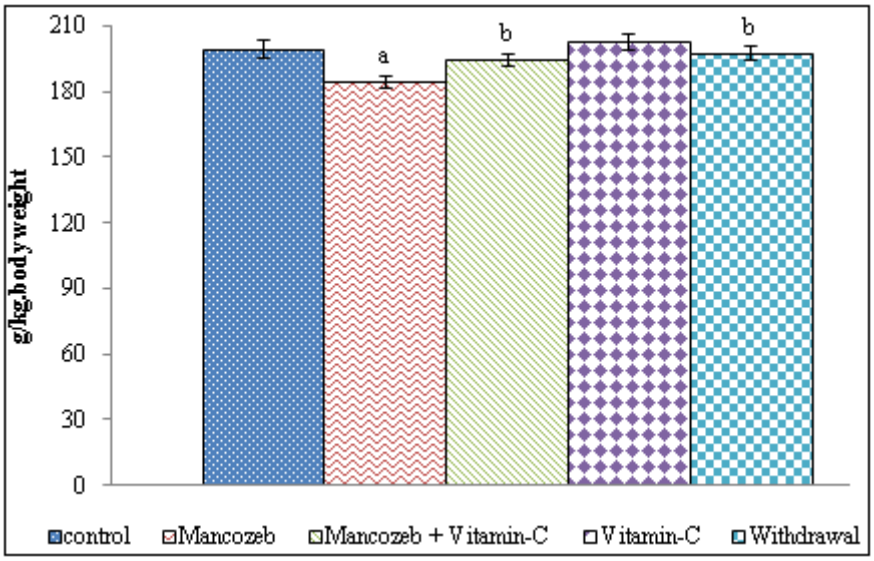

Figure 1: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Body Weight in adult Male rats.

Each value is Mean± SEM of 6 Animals. [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.

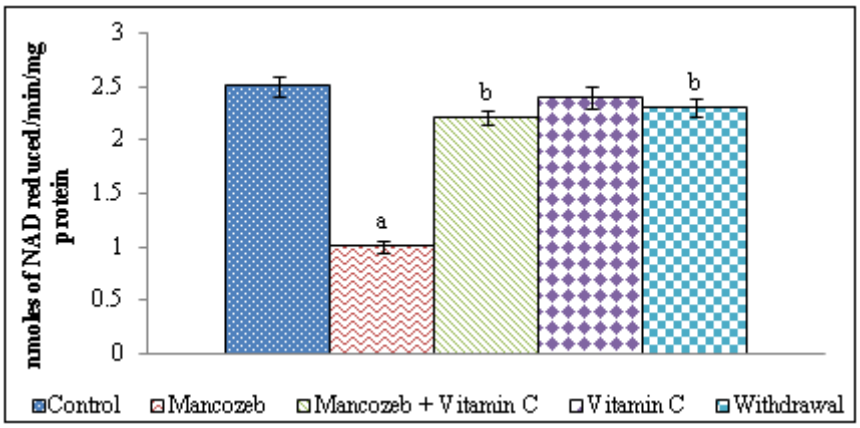

Figure 2: Effect of Mancozeb treatment, Co-administration of Vitamin C with Mancozeb,Vitamin C alone and Withdrawal treatment on 3ß- Hydroxysteroid Dehydrogenase enzymes activities in adult Male rats

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.

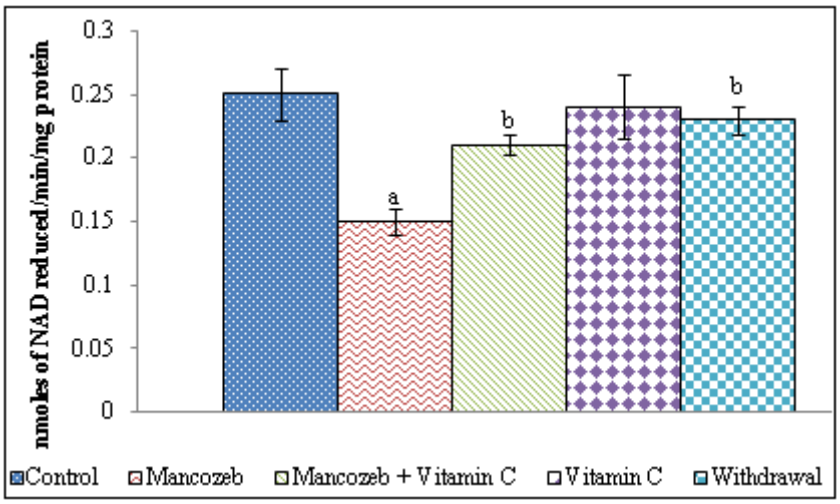

Figure 3: Effect of Mancozeb treatment, Co-administration of Vitamin C with Mancozeb, Vitamin C alone and Withdrawal treatment on 17ß- Hydroxysteroid Dehydrogenase enzymes activities in adult Male rats

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.

3. Results and Discussion

Mancozeb, is one of the organo-metallic dithiocarbamate fungicide and has been shown to be toxic to the reproductive, endocrine and immune systems in different species (Baligar and Kaliwal, 2001; Corsini et al., 2005; Mills et al., 2005). Body weight is an indicator of protein and fat metabolism.

The reduction in body weight may be due to high rate of protein breakdown, which might be needed to fulfill energy requirements during detoxification (Ananthan.G and Kumaran,B: 2013). (Fig: 1) another reason was found anorexia and general weakness, weight loss in animals exposed to Maneb, Zineb and Mancozeb. Ivanavo-Chemishanska (1969)

3β-HSD, which is the key enzyme that catalyses the conversion of pregnenolone to progesterone and 17β-HSD, which is necessary for the formation of testosterone from Androstenedione (Mendis-Handagama, 2000). Present study the results revel that the Mancozeb administration significantly decreased ($p<0.05$) the activities of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) compared to control (Fig: 2). Testicular 3β-HSD and 17β-HSD activities were lowered in the Mancozeb administered rats. The decreased steroidogenic potency in the Mancozeb-treated rats was also observed in serum testosterone titer. The decreased activities of steroidogenic enzymes studied (3β-HSD and 17β-HSD) in the Mancozeb-treated rats may also be due to the increased production of LPO and ROS in the Testis. Co-administration of Mancozeb with Vitamin-C brought back to the normal levels. Vitamin-C alone treatment did not show any significant change in the level of steroidogenic enzymes. However, withdrawal of Mancozeb treatment resulted in restoration of the activities of 3β-hydroxysteroid dehydrogenase and 17β- hydroxysteroid dehydrogenase to control.

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