

ORIGINAL ARTICLE

Effect of Mancozeb on the Specific activities of Testicular Phosphatases and Protective role of Vitamin C in Albino rats

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ABSTRACT

*Mancozeb, a fungicide of Ethylene-bis-dithiocarbamate 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. 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 significantly decreased the activities of Acid Phosphatase and Alkaline Phosphatase in testis of rats and increased the activities in the serum. Co-administration of Vitamin C with Mancozeb restored the activities of these enzymes to normalcy. Vitamin C did not cause any adverse effects on the parameters studied. The toxic effect of Mancozeb was reversible in the withdrawal group. The present study reveals the protective effect of vitamin C on the Mancozeb induced Testicular toxicity in the adult rats.*

Keywords: Mancozeb ; Vitamin C ; Acid Phosphatase ; Alkaline Phosphatase; Testis.

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INTRODUCTION

Mancozeb is an ethylene-bis-dithiocarbamate (EBDC), fungicide used against a wide range fungal disease of field crops, fruits and ornaments [1]. EBDC group is a polymeric complex of 20% manganese with 2-5% zinc salt. 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 [2].

Mancozeb has been shown to produce adverse effects on Skin, liver, kidney, Central Nervous System, Male and Female Reproductive System and chromosomes of bone marrow cells in mice, Rats and Human [3-6]. Many studies have shown that Mancozeb has the ability to cause immuno modulation in workers, such as increased CD19+ cells, decreased CD25+ cells, and tumor necrosis factor, and increased risk of leukaemia [7,8].

Evidences are available to suggest that the Mancozeb has deleterious effects on various aspects of male reproduction. The effects include mean testes weight, total Sperm count, and motility, increased incidence of sperm abnormalities, germ cell depletion and altered testicular functions. However, the information on the toxic effects of this fungicide on the specific activities of Testicular phosphatases is limited. Therefore, the present investigation was undertaken to elucidate the effect of Mancozeb on the activities of phosphatases in the serum and testis of adult albino rats.

MATERIALS AND METHODS

Chemical:

Mancozeb (85% purity) was a gift from the Krishi Rasayn Exports Pvt. Ltd. Hyderabad, India.

Animals

Healthy Male adult albino rats of Wistar strain weighing 200-210 g were housed in 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) (845/ac/04/2004). 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 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 (1/10th of LD₅₀) 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 left untreated for further period of 60 days to observe withdrawal effects.

Collection of tissues

The rats were weighed and sacrificed twenty-four hours after the last treatment, by anaesthetic ether. The testes, epididymis, seminal vesicle, ventral prostate were removed from the adhering connective 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 0.32 M Sucrose solution and the homogenate was centrifuged at 10000× g for 30 min at 4°C. The Supernatant was used for various biochemical assays. Protein was determined according to the method of Lowry, et al., [9]. The activity of Acid Phosphatase was estimated by the method of Tenniswood, et al., [10]. Alkaline phosphate was assayed following the method of Bessey, et al., [11].

Statistical Analysis

Single way Analysis of Variance (ANOVA) was followed to analyse the data according to Zar [12]. If the 'F'-ratio was significant, Student-Neumann-Keul's (SNK) test was followed.

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 [5, 7,8]. Body weight is an indicator of protein and fat metabolism. The effect of Mancozeb at the dose of 300mg/kg body weight for 60 days on the body weight and reproductive organs weight in adult albino rats has been presented in Figs (1-3). Administration of Mancozeb significantly decreased ($p < 0.05$) the body weight, testicular weight and accessory sex organs weights compared to control rats. Co-administration of vitamin-C registered normal body weight and organs weight compared to Mancozeb treated rats. Vitamin C given alone did not have any significant change in the body weight and organ weight. However, the withdrawal of Mancozeb, restored the normal weight in the body and organs. The reduction in body weight may be due to high rate of protein breakdown, which might be needed to fulfil energy requirements during detoxification. Ivanavo-Chemishanska [13] found anorexia and general weakness in animals exposed to Maneb, Zineb and Mancozeb. Probably, this may be another reason for weight loss in animals exposed to Mancozeb in the present study.

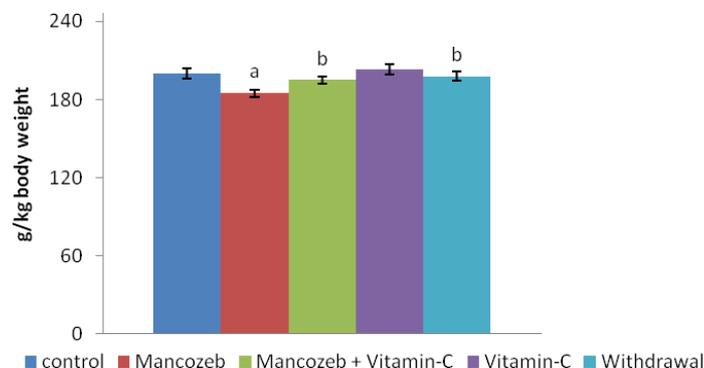
The reduction in the testicular weight observed in Mancozeb treated rats suggests the degenerating capacity of Mancozeb. Reduction in the weight of the testis could be due to inhibition of seminiferous tubule fluid formation and loss of germ cell by direct inhibition on spermatogenesis. Reduction in testicular weight is in agreement with earlier studies on rats [14] and Swiss mice [15]. It is well established that the structural and functional integrity of the Male accessory sex glands are androgen dependent [16-18]. In the present study, the weight of Male accessory sex organs were decreased significantly which might be due to decreased bioavailability androgens and estrogenic and antiandrogenic activities of Mancozeb [19].

Mancozeb treatment for 60 days caused significant decrease ($p < 0.05$) in the specific activities of Acid Phosphatase and Alkaline Phosphatase in the testicular tissue of rats and increased the activities in the serum. (Figures: 4-7). However, Co-administration of Vitamin-C with Mancozeb maintained the normal activity of Phosphatases. Vitamin C alone treated rats didnot show any change in the activities of phosphatases in both serum and tissue. Withdrawal of Mancozeb restored the normal activities of these enzymes in the serum and testis of the adult rats.

Alkaline Phosphatase is a 'Marker' enzyme for plasma membrane and endoplasmic reticulum. It has been shown that the alkaline Phosphatase is involved in the mediation of membrane transport [20] and transphorylation [21]. The decrease in the activity of alkaline Phosphatase in the testis following the repeated administration of mancozeb could be attributed to either leakage of the enzyme into the extracellular fluid as a result of the disruption of the ordered lipid bilayer of the membrane or inhibition

of the enzyme activity by this fungicide. Similar depletion in alkaline phosphatase was also observed in the testis of rats treated with mancozeb [14] and endosulphan [22] who reported that the reduction in the activity of this enzyme is due to the decreased metabolic activities.

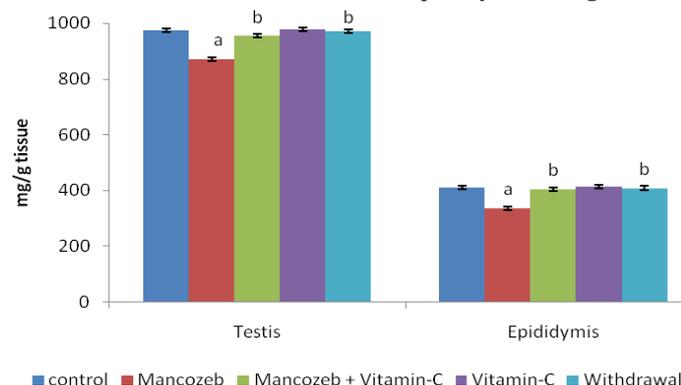
Figure 1: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Body Weight in adult rats.



Each value is Mean ± SEM of 6 Animals.

a and b represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively.

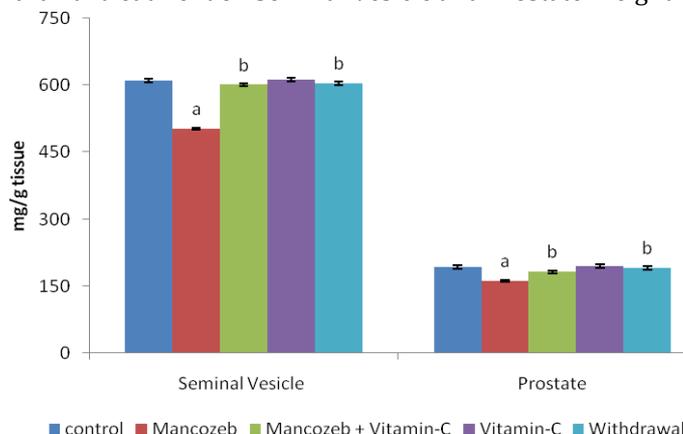
Figure 2: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Testis and Epididymis Weight in adult rats.



Each value is Mean ± SEM of 6 Animals.

a and b represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively.

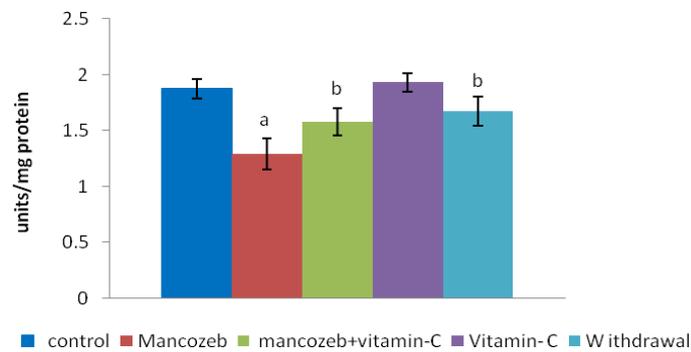
Figure 3: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Seminal Vesicle and Prostate Weight in adult rats.



Each value is Mean ± SEM of 6 Animals.

a and b represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively.

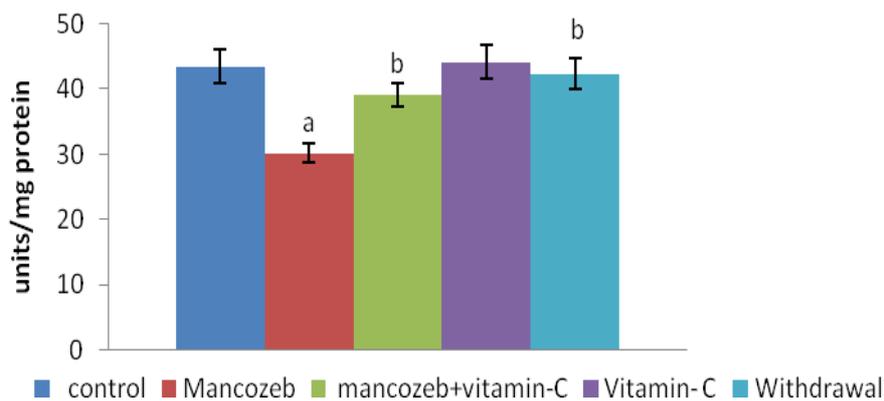
Figure 4: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Alkaline Phosphatase activity in Testis of the adult rats.



Each value is Mean± SEM of 6 Animals.

a and b represent statistical significant at P<0.05 compared with Control and Mancozeb, respectively.

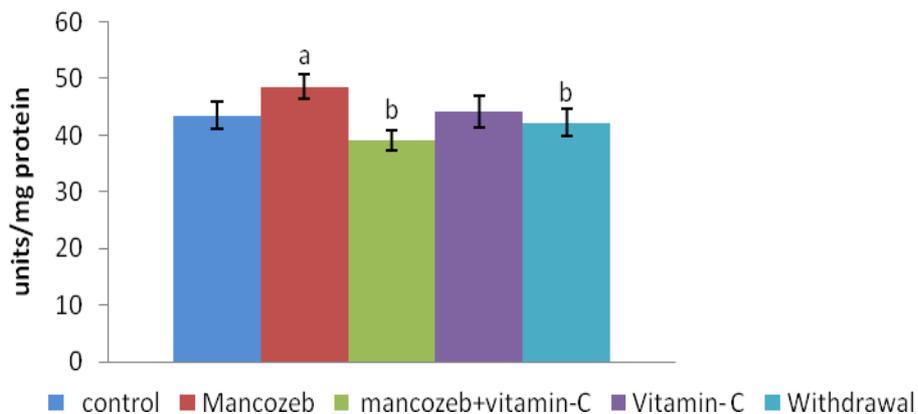
Figure 5: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Acid Phosphatase activity in Testis of the adult rats.



Each value is Mean± SEM of 6 Animals.

a and b represent statistical significant at P<0.05 Compared with Control and Mancozeb, respectively.

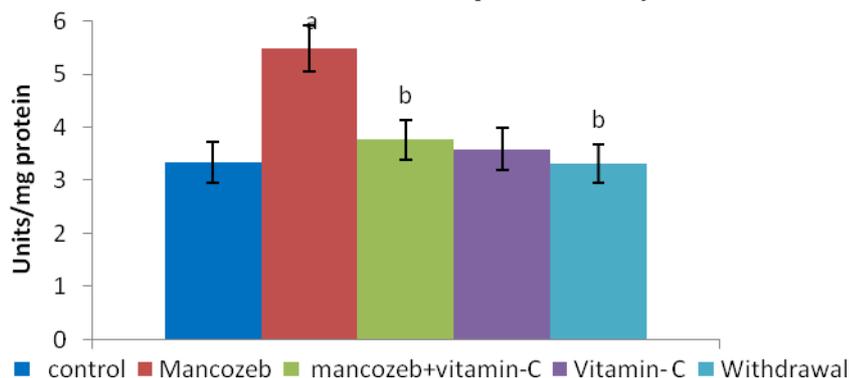
Figure 6: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Alkaline Phosphatase activity in Serum of the adult rats.



Each value is Mean± SEM of 6 Animals.

a and b represent statistical significant at P<0.01 Compared with Contro and Mancozeb, respectively.

Figure 7: Effect of Mancozeb treatment, Co-administration of Vitamin C With Mancozeb, Vitamin C alone and Withdrawal treatment on Acid Phosphatase activity in Serum of the adult rats



Each value is Mean \pm SEM of 6 Animals.

a and b represent statistical significant at $P < 0.05$ Compared with Control and Mancozeb, respectively.

Acid phosphatase a 'marker' enzyme for the lysosomal membrane. It is used to estimate interference with catabolic and autophagic process in the tissues. The decreased activity of acid phosphate in the testis of mancozeb treated rats may be attributed to leakage of the enzyme from the lysosomes into the extracellular fluid. Further the depletion in the activity of acid phosphatase is due to the inhibitory effect of Mancozeb there by affecting the function of the testis. The results of the present study could therefore indicates that the Mancozeb induced cell damage resulted in the release of phosphatases in to the blood stream, hence reducing its level in the testis and increasing level in the serum. This effect is similar to the effect of other pesticides like Quinalphos [23] and Methyl parathion [24].

Co-administration of Vitamin C with Mancozeb resulted in complete prevention of the deleterious effect of Mancozeb in body weight, and the reproductive organs weight studied in the present investigation. Vitamin C is a naturally occurring antioxidant found in both animals and plants [25]. Additionally, vitamin C is an excellent radical scavenger [26, 27] and it may nullify the possible spermicidal and genotoxic effects of various free radicals [28] that appear during cellular metabolism of pesticides [29]. The restoration of activities of Acid and Alkaline Phosphatase 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 levels of pesticide genotoxicity [30].

Thus, the present study reveals that the treatment of Mancozeb is capable of inducing adverse effects on the activities of Phosphatases in the testis of adult rats. However, Co-administration of Vitamin C with Mancozeb imparts protective effect on the testicular phosphatases. It is interesting to note that almost all parameters that were affected by Mancozeb treatment were restored to normalcy upon withdrawal of Mancozeb treatment which clearly indicates that these effects are transient and reversible.

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