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SPERMATOTOXIC EFFECT OF MANCOZEB IN ADULT RATS

Zoology

KEYWORDS: Mancozeb, Testis, Epididymis, sperm count, motility, viability and abnormalities.

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ABSTRACT

Endocrine disrupting chemicals (EDC) are an heterogeneous group of substances present in the diet and environment able to modify endocrine homeostasis, in particular steroid and thyroid hormones: therefore reproductive health and developing life stages are the most susceptible targets. 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 effect of Mancozeb induced reproductive toxicity in adult albino rats. 90 days old adult male Wister rats (Rattus norvegicus) were exposed to Mancozeb at the dose of 300 mg/kg body weight, orally for 60 days. Dosages represent 1/10 LD 50 of Mancozeb, respectively. After the treatment period, animals were euthanized, testis and accessory sex organs were removed and weighed. Epididymis were quickly, encapsulated and utilized for the estimations of sperm count, motility, viability and abnormalities. Mancozeb treatment resulted in a significant decrease in body weight, the epididymal sperm count, motility and viability as compared to control animals. Withdrawal of Mancozeb treated rats restored the Organs Weight, normal sperm morphology, sperm count, motility and viability. The present study reveals that the effect of Mancozeb on Spermatological toxicity in the Adult Male Rats.

INTRODUCTION

Endocrine disruptors (EDs) have been described as exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of the natural legends responsible for maintaining homeostasis and regulating body development.(Mostafa et al., 2007). Endocrine disrupting chemicals (EDC) interact with nuclear or with enzymes responsible of synthesis or transportation of hormones they include persistent contaminants used in plant and /or animal production (e.g., dicarboxmides, triazoles) and compounds found in industrial as well as consumer products (e.g., bisphenol A, several phthalates, polybrominated flame retardants)(Mantovani et al.,2002).

The reproductive effect of carbamate pesticides are of immense important in the field of toxicology. Carbamates are chosen on the basis of their properties of biodegradable with low mammalian toxicity. Mancozeb (Manganese ethylene bis dithiocarbamate polymeric complex with zinc salt) a carbamate form class of fungicide used against a verity of foliar fungal diseases and seed treatment, it has significance over other carbamates. Mancozeb shows its biological effect through it's metabolites like (ETU) and carbon disulfide (CS2)(O'Neil and Marshal,1984). Carbon disulfide causes significant marked degenerative changes in testicular tissue, affects spermatogenesis and also epididymal alterations(Patel Kumar et al.,1999).

Exposure to mancozeb causes hormocytic types of anemia, significant decrease in blood glucose and globulin levels and significant pathological changes were observed in Skin, Thyroid, Liver, Kidney, Spleen and heart showed congestion with slight enlargement and central nervous system revealed few pctechial hemorrhages (Hore et al., 1997: Szepvolgyi et al., 1989; Kackar et al.,1997). It had also been reported as tumor initiator and promoter on mouse skin (HWO, 1982: Hayes Jr WJ et al., 1991: Shukla et al., 1990 : Lisi.P et al., 1985). Mancozeb has produced significant enzymatic changes in the activities of various enzymes (Kackar et al.,1999). It was found to produce chromosomal aberrations in wister rats (Georgiam et al., 1983, in mice (Kriti Tripathi et al., 2011). Recently, Mancozeb has been shown to produce significant toxicological effect on male and female reproductive system and hormonal profiles and imbalance in animals and humans(C.Joshi et al.,2005: S.A.Sakar et al.,2009: Parimala and Kliwal,2005: Mahadevasmai et al., 2000: Baligar and Kaliwal, 2001: Bindali and Kaliwal 2002). The present work was conducted to study the adverse effect of Mancozeb fungicide on Spermatological effect in Adult Male 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.

Animal and treatment

Adult male albino Wister rats (Rattus norvegicus) of an average body weight 200 – 210 gms have been employed for experimentation. The animals were purchased from the Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were kept in clean polypropylene cages covered with chrome plate grills and maintained at (24± 2°C) temperature for at least one week before and throughout the experimental work. 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)(Reg.No.845/ac/04/CPCSEA:No:101/KMCPGS/IAEC/2009-2010/Dated 12-05-2010).The animals were maintained on a standard diet and water was available ad libitum. Animals were divided into 3 groups:

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 daily for 60 days, orally.

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

The dose and duration of Mancozeb was selected according to the previous investigation carried out by (Saker et al. 2005) they have standardized this as the optimal dose for bringing about Testicular and Spermatological effects. The same dose has been selected for the present study.

Collection of tissues:

saline repeatedly and were weighed and kept on ice at 4°C. The epididymis was used for spermatological The rats were weighed and sacrificed twenty-four hours after the last treatment, by anesthetic ether. The testes, epididymis, seminal vesicle and ventral prostate

were removed and cleaned of fat and adhering tissues, washed in cold physiological studies.

Spermatological studies: Epididymal sperm were collected by the method of Gray, *et al.,* (1989).

Sperm Viability tests: Sperm viability test was done by the method as described in the WHO Laboratory Manual (1999).

Epididymal Sperm Motility: Motility of epididymal sperm was evaluated by the method as described by Linder, *et al.,* (1986) and Cooke, *et al.,* (1991).

Epididymal Sperm Counts: Epididymal sperm were counted by the method as described in the WHO Laboratory Manual (1999).

Sperm concentration = No. of sperm counted x Multiplication factor.

Statistical analysis

All the data were analyzed using students t-test and data were expressed as mean ± SD for six animals per group. The value of P<0.05 was considered on significant against control.

Results and Discussion

Body weight and organ weights:

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 effect of Mancozeb at the dose of 300 mg/kg body weight for 60 days on the bodyweight and reproductive organs weight in adult albino rats has been presented in Figures (1-5). Administration of Mancozeb significantly decreased (p<0.05) the body weight, testicular weight and accessory sex organs weights compared to control rats. However, the withdrawal of Mancozeb, restored the normal weight in the body organs. The reduction in body weight may be due to high rate of protein breakdown, which might be needed to fulfill energy requirements during detoxification. Ivanavo-Chemishanska (1969) 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 (Joshi,et al., 2005) and Swiss mice (Ksheerasagar and Kaliwal, 2003). It is well established that the structural and functional integrity of the Male accessory sex glands are androgen dependent (Mann, 1951; Willams-Ashman, 1972; Luke and Coffey, 1994).

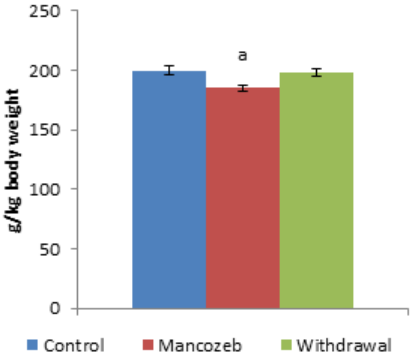

Figure 1: Effect of Mancozeb treatment on Body weight in adult male rats.

Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with control and Mancozeb.

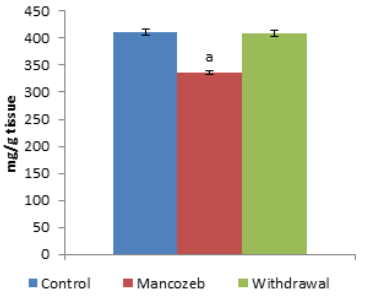

Figure 2: Effect of Mancozeb treatment on Testicular Weight in adult male rats.

Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

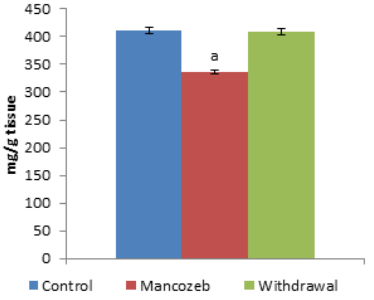

Figure 3: Effectof Mancozeb treatment on Epididymis Weight in adult male rats.

Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

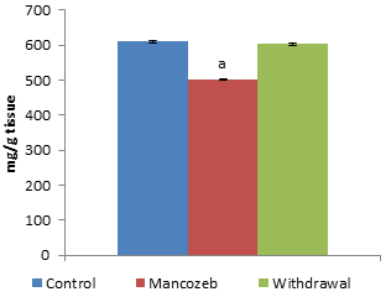

Figure 4: Effect of Mancozeb treatment on Seminal Vesicle Weight in adult male rats

Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

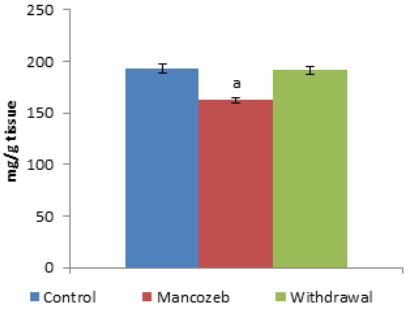

Figure 5: Effect of Mancozeb treatment on Ventral Prostate Weight in adult male rats.

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Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

Plate a to k Different forms of sperm abnormalities in Male rats

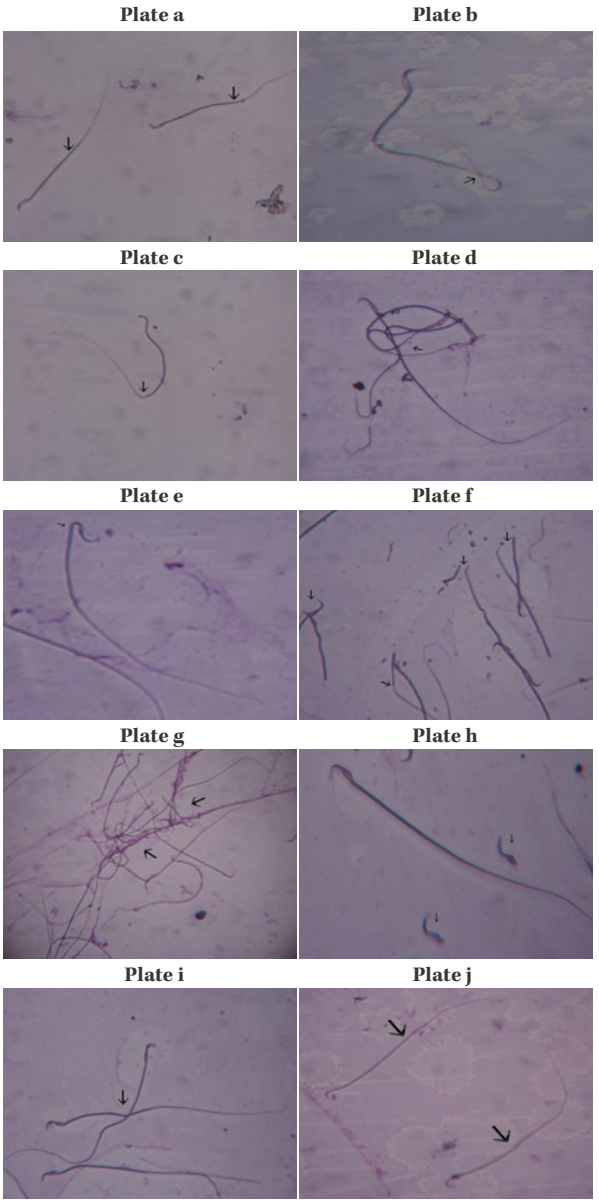

Plate a: Normal sperms with Head, Body, Tail.
Plate b : Curved or Coiled Tail sperm.
Plate c: u shaped sperm. Plate d : Q shaped sperms.
Plate e: Coiled bent Head sperm.
Plate f : Detached sperms.
Plate g : Agglutinated sperms Plate h: Detached Head sperm.
Plate i & j: Withdrawal of Mancozeb, Normal sperms with Head, Body & Tail.

Figure 6: Effect of Mancozeb treatment on Epididymal Sperm Count in adult male rats

Each value is Mean ± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb

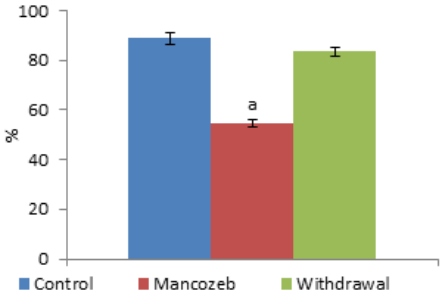

Figure 7: Effect of Mancozeb treatment on Epididymal Sperm Motility in adult male rats.

Each value is Mean± S E M of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

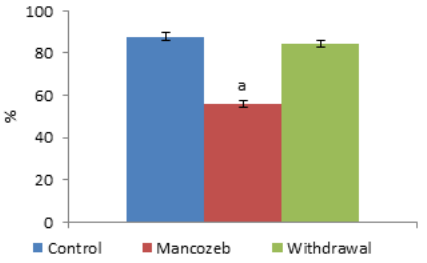

Figure 8: Effect of Mancozeb treatment on Epididymal Sperm Viability in adult male rats.

Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

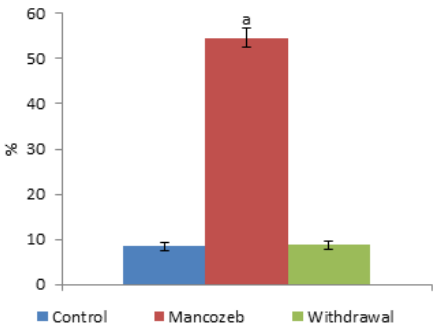

Figure 9: Effect of Mancozeb treatment on Epididymal abnormal Sperm in adult male rats.

Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

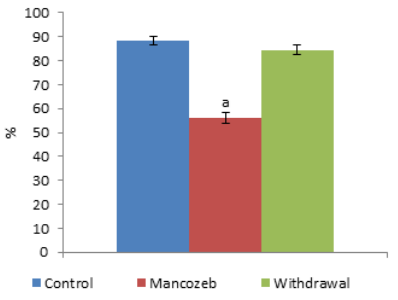

Figure 10: Effect of Mancozeb treatment on Epididymal Normal Sperm in adult male rats.

Each value is Mean± SEM of 6 Animals. a represent statistical significant at P<0.05 Compared with Control and Mancozeb.

Spermatological effect of Mancozeb:

Evidences are available to suggest that the pesticides and fungicides have the potential to disturb the endocrine function, leading to impaired reproduction capacity and other toxic effects on sexual differentiation, growth and development (McLachlan, 2001). Administration of mancozeb at a dose of 1000 mg/kg body weight for 35 days in males of 6 to 8 weeks old Swiss albino mice revealed that this fungicide produced a significant decrease in sperm count, along with a marked increase in the frequency of abnormal head sperm (Khan and Sinha, 1996). Carbendazim, also a fungicide caused the deleterious effect on sperm morphology and increase incidence of abnormal sperm in adult rats (Nakai, *et al.,* 1992; Akbarsha, *et al.,* 2001). In the present investigation, The effects of Mancozeb administration on the sperm abnormalities in adult albino rats has been presented in (Figures 6-10). In control rats, about 88% of the sperm possessed normal morphology as described above whereas about 12% sperm possessed abnormalities of which head alone, curved tail and attached sperm predominated. In the rats treated with mancozeb for 60 days, only about 30% were normal in morphology, the remaining abnormalities sperm. Epididymal sperm density may be due to alteration in androgen metabolism. The physiological and biochemical integrity of epididmis are dependent on androgens (Brooks, 1979). The 80% negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of caudal epididymis (Joshi et al., 2003). Fungicides induce inhibition of glycolytic enzymes, which may affect the maturational process of spermatozoa and their motility. Inhibition of glycogen synthesis eventually decreases spermatogenesis process (Desta, 1994). The effects of Mancozeb administration on the sperm abnormalities were the detached head , head facing to the flagellum, agglutinated sperms (Two or more sperm attached among themselves for short or long distances, at one or more points).Curved or coiled tail, detached sperm head, body and tails. Further, some of the sperm had an abnormal morphology of 'Q' and 'u'-shaped sperm attached it has been presented in (plate a – j). The Mancozeb treatment resulted in a significant decrease in the epididymal sperm count, motility and viability as compared to control animals. However, The Withdrawal of Mancozeb treated rats restored the normal sperm morphology, sperm count, motility and viability.

Conclusion

These findings can suggest us to conclude that the effect of Mancozeb induced Spermatological toxicity remarkable sperm cells degeneration lowed the sperm quality, quantity and sperm abnormalities. However, these effects are transient and reversible upon withdrawal of Mancozeb treatment.

Acknowledgement

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