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Full Length Research Paper

The effect of Maneb on implantation, fecundity rate and the thyroid activity in the rabbit

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The fungicide Maneb is a member of the family of dithiocarbamates that is used in the control of the fungal diseases of plants. The purpose of this work is to examine the effect of Maneb on implantation and thyroid activity at doses of 5, 10 and 50 mg/kg/day by gavage for 10 days in the domestic female rabbit, *Oryctolagus cuniculus*. The rabbits were sacrificed on the 11th day of pregnancy. The results indicate an increase in the body weight in the treated female. An increase is also observed in the weight of liver in the treated females with a darker color. There is 50% of inhibition of implantation in the group treated with the higher dose (50 mg/kg/day) when compared with control rabbit. The inhibition of implantation by Maneb may be to due to hormonal imbalance.

Key words: Maneb, implantation, fecundity, thyroid, rabbit.

INTRODUCTION

As a chemical family, the ethylenebisdithiocarbamate (EBDC) pesticides are regarded as fungicides with a wide range of uses including many fungal diseases of vegetables, fruits, and field crops. They have been on the market since the 1930s and 1940s. The class includes Nabam, Maneb, Mancozeb, Metiram and Zineb. The organometallic fungicide, Maneb, is widely used in agriculture to protect against a wide spectrum of fungal diseases many fruits, vegetables and nuts. Maneb has a low acute toxicity but high or repeated exposures may interfere with gonads and thyroid functions (Bharati and Basappa, 2002).

Few studies have been carried out on the chronic toxicity of dithiocarbamates containing heavy metals such as manganese (Mn) and zinc (Zn). Ethylene thiourea (ETU) is major metabolite of ethylenebisdithiocarbamate fungicides which has been reported to be carcinogenic, teratogenic, and mutagenic in experimental animals (Teramoto et al., 1975; Larsson et al., 1976; Tanaka et al., 1995). After chronic exposure, Maneb has been shown to produce adverse effects in fertilization, damage in the central nervous system (Edward et al., 1991; Sitting and Mane, 1991; Kurinni and Kandratenko, 1992; Kachar et al., 1997). It also has been shown that Mancozeb affects pregnancy in female rats and induces gonadal toxicity in male rats (Kachar et al., 1997; Neyko et al., 1974; Kaskevich et al., 1981).

There are no reports on the effect of Maneb in site implantation in rabbits. The study of Bharati and Basappa (2002) investigated the anti-implantation effect of Mancozeb in mice. Thus, the aim of the present study was to evaluate the effects of Maneb on the fecundity, site implantation and the function of thyroid in rabbit (*Oryctolagus cuniculus*) after chronic exposure.

MATERIALS AND METHODS

Animals

Twenty four pregnant female rabbits of initial body weight of 1100 - 2500 g were divided into three treated groups (six animals for each group) and six females were used as control. They were acclimatised to laboratory conditions for 15 days. The female were mated with non-exposed male. The criterion used to identify mated fe-

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Table 1. Organ weight in the pregnant rabbit after treatment.

Groups	Organ's weight (g); mean ± SD, (n = 6)			
	Heart	Liver	Kidney	Spleen
Control group	4.19±0.92	44.48±12.14	4.85±1.22	0.96±0.84
Treated group with 5 mg/kg	3.22±0.49 *	41.63±13.14 *	3.61±0.54 ns	0.62±0.16 *
Treated group with 10mg/kg	4.63±1.02ns	47.48±4.94 ns	4.64±0.37 ns	0.94±0.20 ns
Treated group with 50 mg/kg	4.02±0.69*	65.58±50.89 *	5.09±1.24 *	1.64±1.38*

males was the presence of spermatozoa in the vagina (0 day of gestation). All pregnant females were individually housed in the special cages at room temperature in the favourable conditions.

Administration

In this experiment, the Maneb (Dicotan M22) 80% pure wettable powder was used. A suspension in water (1 liter) was prepared in order to obtain 5, 10 and 50 mg/kg body weight. by gavage. The animals were divided in 4 groups (6 females in each group); control group, and treated groups with 5, 10 and 50 mg/kg/d, respectively. The animals were weighed every day for 10 days (treatment period) and sacrificed on the 11 day of pregnancy. The blood was collected in EDTA coated tubes. Plasma collected was used for determination of biochemical parameters (cholesterol, triglycerides, glucose) and thyroxin (T_4). The liver, kidney, heart and spleen of each animal were weighed before the histological study. The number of the embryonic implantations was observed for each female (of each group). The rate of fecundity was calculated with the follow equation as described by Vaissaire (1977):

$$\text{Rate fecundity (\%)} = \frac{\text{Number of mated female}}{\text{Number of pregnant female}} \times 100$$

Biochemical assays

Plasma glucose, triglycerides and cholesterol levels were measured as described by Trinder (1969). Thyroxin hormone was measured following the ELISA method.

Statistical analysis

All results were expressed as mean ± SD. and analyzed using Student t-test with Minitab program (version 13), between the control and each treated group.

RESULTS

Body weight

The treated female had less activity especially those treated with 50 mg/kg/d. At the end of the period treatment, two rabbits died in the treated group with 50 mg/kg. Zones of alopecia were noted in the second and third treated groups on the last day of the first week of the treatment. We also observed that treated animals consumed much more food then the control group. A significant increase in the body weight was observed in the treated rabbit as compared with the control group.

Weight of organs

An increase in the weight of the liver of rabbits given Maneb and a darker color of this organ was observed in the treated animals as compared with the control group. The results revealed a perturbation in the weight of the heart, kidney and spleen in the treated rabbit (Table 1).

Biochemical parameters

Table 2 demonstrated a very significant increase in plasma glucose levels was observed in treated groups compared to controls. An increase was noted in triglycerides concentrations of animals treated with Maneb. The results indicated, also a significant increase in plasma cholesterol concentrations in treated rabbits.

Fecundity number of site implantation

The results indicate that the mated control females became pregnant and showed normal embryonic implantation sites (Figures 1 and 2). Treatment with Maneb for 10 days of pregnancy caused a decrease of site implantations in the mated females, especially with doses (10 and 50 mg/kg) as shown in Figures 4 and 5 compared with the control (Figure 3). Maneb was also found to affect the rate of fecundity as shown in Table 3.

DISCUSSION

The results demonstrated clearly a significant increase in the body weight, especially in the treated group with 50 mg/kg after 8 days of treatment. Such a response might be due to the effect of the dithiocarbamate on the thyroid gland, by reducing the secretion of thyroxin (T4). It has been known for a long time that thyroxin administration

Table 2. Variation of biochemical parameters (g/l) after treatment with Maneb for 10 days of pregnancy in the rabbit (mean ± SD, n = 6).

Groups	Concentration (g/l) (n = 6)		
	Glucose	Triglycerides	Cholesterol
Control group	0.50±0.14	1.74±0.37	2.54±0.22
Treated group with 5 mg/kg/d	1.60±0.22**	1.92±0.88ns	3.77±1.07*
Treated group with 10 mg/kg/d	1.70±0.60**	2.46±0.83*	3.13±1.45ns
Treated group with 50 mg/kg/d	1.88±1.08**	3.00±0.76**	3.21±0.60*

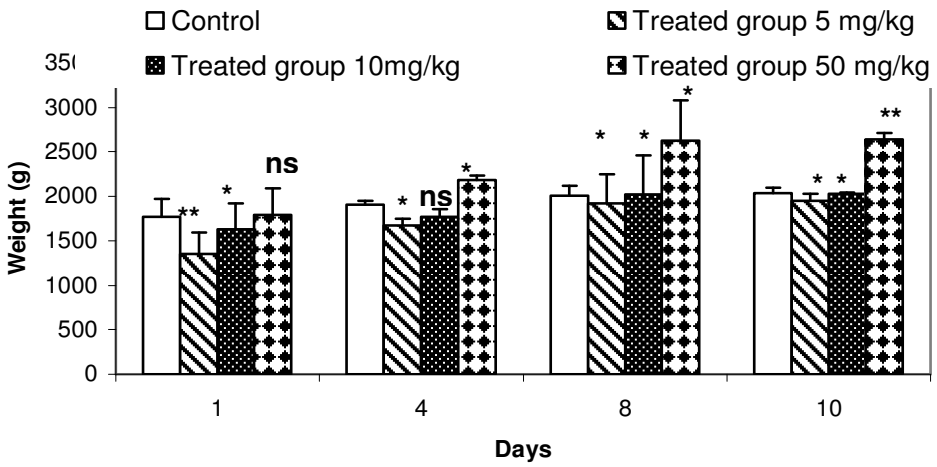

Figure 1. Variation of the body weight (g) in the rabbit after 10 days of pregnancy after period treatment. (Mean ± SD, n = 6).

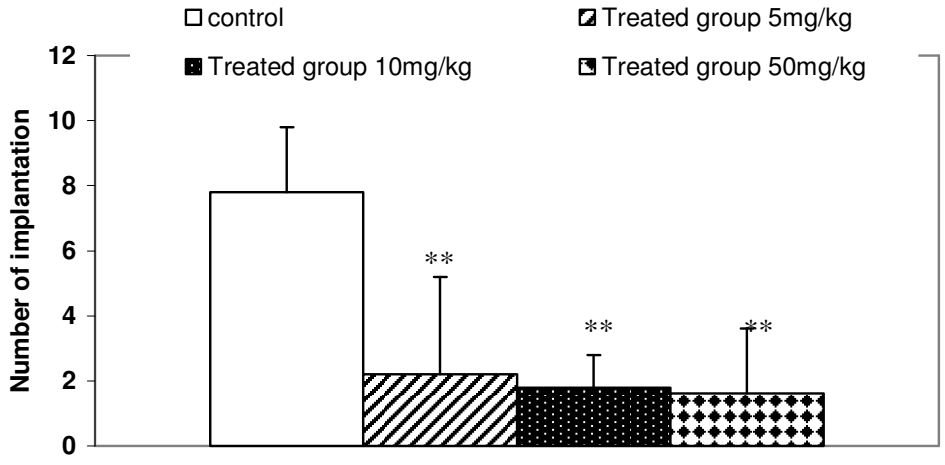

Figure 2. Variation of the number of embryonic implantations in the various groups of rabbits (mean ± SD, n = 6).

causes a reduction in body weight, whereas the ablation of thyroid causes, on the contrary, an increase in body weight in birds (Nicholls et al., 1988). The effect of high doses of Maneb (2500 mg/kg), on the thyroid activity of rats was reported by previous investigations made by Worthing and Walker (1983). Besides, similar studies revealed that dithiocarbamates affects thyroid activity, by decreasing the secretion of TSH, causing a reduction in the circulating (T4) and inducing hypothyroïdy (Jeffrey et al., 2001). These results could explain not only the in-

Figure 3. Uterus of pregnant control rabbit showing normal implantation.

Figure 4. Uterus of pregnant rabbit treated with Maneb (10 mg/kg/d) for 10 days, showing less implantations (IM).

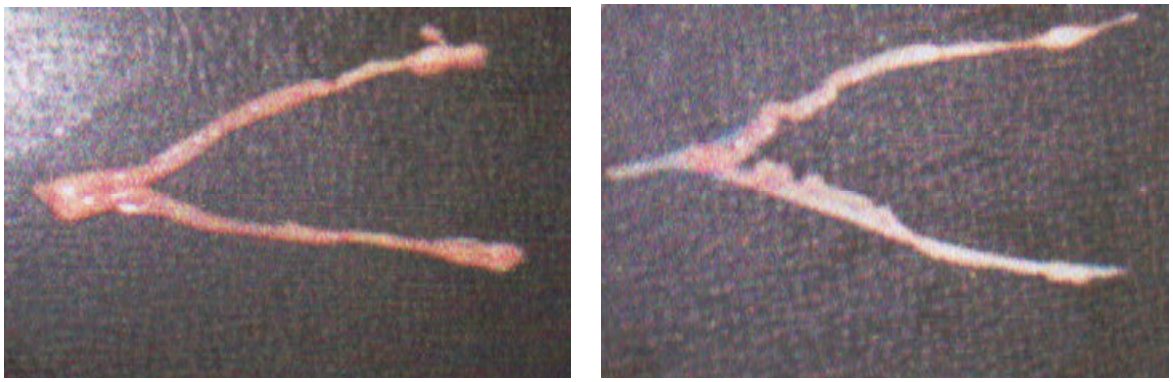

Figure 5. Uterus of pregnant mice treated with Maneb for 10 days (50 mg/kg/d), showing no implantation (IM).

Table 3. Concentration of thyroxin hormone and fecundity rate in the treated and control female rabbits (mean ± SD, n = 6).

Groups	Control	Treated groups (mg/kg body weight)		
		5	10	50
Concentration of thyroxin (T_4) (ng/dl)	1.16±0.016	0.93±0.019**	0.85±0.012***	0.73±0.014***
Fecundity (%)	83.33	66.66	50	33.33

crease in body weights of the animals but also the increase in the concentrations of triglycerides and glucose and cholesterol (WHO, 1988; Kachar et al., 1997).

The results obtained in the present study suggest that the treatment with the Maneb especially with the doses 10 and 50 mg/kg for 10 days of pregnancy period in the rabbit caused a significant decrease in the number implantation. Similar results have been reported that the implantation was reduced with Clhlordecone (Johnson et al., 1988).

(Pinkston and Uphouse, 1988) have reported that when DDT is administered at the time of mating or early after fertilization results in the loss of pre-implanted embryo. It has been revealed that organophosphate pesticides affect implantation, gestation, foetal growth, and abnormalities in mammals and development in the birds (Khan, 1981; Wyettenbach and Thompson, 1985).

It has also been reported that mancozeb produced significant toxicological effects on thyroid and had adverse effects on fertility but not on embryo toxicity (Edward et al., 1991; Kurinni and Kandratenko, 1992; Kachar et al., 1997).

The treatment of Maneb after medium exposure with high dose (50 mg/kg/d) caused an inhibition of implanttation which may be due to an imbalance of gonadotrophin secretion, via a central nervous system mechanicsm, as it was observed in the rats, following administration of dithiocarbamate (Goldman et al., 1997).

It has also been reported that the toxic agent may act directly on the gonadotrophins to alter indirectly the pituitary cell which is responsible of the secretion of the FSH and LH (Dickarson et al., 1992).

In conclusion, we think that the repeated administration of Maneb with the used doses (5, 10 and 50 mg/kg/d) by gavage may cause structural and functional disorders in the hormonal system.

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