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EFFECT OF MARSHAL (CARBOSULFAN, METHYL CARBAMATE) ON REPRODUCTIVE SYSTEM AND THYROID GLAND OF MALE RATS

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SUMMARY

Oral administration of Marshal (carbosulfan, methyl carbamate) in doses of 4.20 and 10.50 mg/kg b.wt. daily for 65 successive days significantly increased the weight of testes and decreased the weight of prostates and epididymes. Epididymal sperm characters including sperm motility and concentration were reduced, while the sperm abnormalities were increased. Moreover, the activity of testicular enzymes lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) were increased, while the activity of acid phosphatase was decreased. Marshal increased the weight of thyroid glands and the plasma level of thyroid stimulating hormone (TSH), while triiodothyronine (T3) and thyroxine (T4) were reduced. Regarding some biochemical parameters, Marshal decreased the blood glucose level and reduced the plasma activities of aspartate amino-transferase (AST) and alanine amino-transferase (ALT), while total cholesterol was increased. Moreover, Marshal induced histopathological alterations in testes and livers. Thus, Marshal has been shown to induce marked structural and functional changes in reproductive system and thyroid glands of male rats.

INTRODUCTION

Carbamates are part of a large group of synthetic pesticides that have developed in the last 40 years. Carbamates are commonly used in agriculture as insecticides, herbicides, fungicides, and nematocides and due to its wide spread use, contamination of food, water, and air has become imminent and consequently adverse health effects are inevitable in humans, animals, wildlife and fish (Gupta, 1994). Previous studies of a structurally similar carbamates; Carbendazim (Nakai et al., 1995 ; Nakai and Hess, 1997) , Benomyl (Hess et al., 1991), Carbofuran (Pant et al., 1995A ; Pant et al., 1997) and Mancozeb (Kackar et al., 1997A) showed adverse effects on

reproduction in rats. It is well known that, the thyroid glands play an important role in the synthesis, storage and secretion of thyroid hormones necessary for normal growth, development and body metabolism. The thyroid gland has also been shown to be a target organ for environmental chemicals including carbamates, which induce thyroid morphological and functional alterations (Hosokawa et al., 1992 and Kackar et al., 1997B). Therefore, the present study was performed to verify the effect of Marshal on reproductive system, thyroid gland and some biochemical parameters in male rats.

MATERIALS AND METHODS

Experimental Materials:-

Marshal;Carbosulfan{2,3-dihydro-2,2-dimethyl-7 -benzofuranyl}(dibutylamino) thio]} methyl carbamate. The empirical formula is C20H33O3SN2 and molecular weight is 381. It is formulated in liquid form of 25% WP with active ingredient 99.8% (FMC Corp., Agricultural Chemical Group, Philadelphia). It was obtained from Central Agricultural Pesticide Laboratory.

Experimental design :-

Fifteen mature male albino rats (150 -175 g) were allocated into three groups of five rats each.. The first group was kept as control and given 0.2 ml saline for 65 successive days by stomach tube . The second and third groups were given marshal orally by stomach tube in doses of 4.20 and 10.50 mg/kg b.wt daily for 65 successive days, respectively (equivalent to1/50 and 1/20 of LD_{50}) (Hall et al., 1990) .

Sampling :-

After 65 days, the rats were weighed and blood samples were taken by orbital sinus puncture on ethylene diamine tetra-acetate (EDTA) and plasma was separated for hormonal and biochemical studies. Then the rats were sacrificed for studying the reproductive system, thyroid gland and histopathology of testes and liver.

Data collection techniques :-

Reproductive system:-

Testes, seminal vesicles, prostate glands and epididymes were dissected out and weighed in relation to their body weights. The epididymal sperm cell count, motility and abnormalities were performed as described by Bearden and Fluquary (1980). One testis from each animal was homogenized according to the method of Hodgen and Sherins (1973) for determination of the following testicular enzymes; acid phosphatase according to the method of Babson and Read (1959) using kits (Quimica Clinica Aplicada, Spain), alkaline phosphatase (Roy, 1970) using kits (Bio Analytica, USA). lactate dehydrogenase (Buhl and Jackson, 1978) using calorimetric kits (Stanbio Lab, USA) and gamma glutamyl transferase (Szasz, 1969) using kits (Quimica Clinica Aplicada) were performed.

Thyroid glands:-

The thyroid glands were removed and weighed

individually for calculation of their relative weights. Plasma levels of triiodothyronine (T_3) and thyroxine (T_4) (Britton et al., 1975) and thyroid stimulating hormone (TSH) (Jackson, 1982) were determined by solid-phase 125I radioimmunoassay by Coat-A-Count Kits (Diagnostic Products Corporation, Los Angeles).

Biochemical studies :-

Blood glucose was determined according to Caraway (1976) using enzymatic calorimetric test; and total plasma cholesterol by the method of Allain et al. (1974). Plasma total protein was determined according to Doumas et al. (1981) using biuret reaction method (Bio Merieux kits) and plasma albumin according to Doumas and Biggs (1972) using calorimetric kits. Plasma transferases; aspartate amino-transferase (AST) and alanine amino-transferase (ALT) activities were determined as described by Reitman and Frankel (1957) using kits of Diamond Diagnostics.

Histopathological studies:-

One testis and the liver from each rat were fixed in 10% formol-saline and stained with Harris haematoxylin and eosin for histopathological studies (Luna ,1968).

Data Analysis :-

Data was subjected to statistical analysis according to the procedures reported by Snedecor and Cochran (1980); and " t " test was performed to evaluate the difference between mean values of the treated group and those of control group. Values are expressed as mean ± S.E.

RESULTS

Marshal increased the weight of testes and decreased the weight of prostates and epididymes, while the weight of seminal vesicles was not changed (Table 1). Regarding the epididymal sperm characters, marshal at both doses decreased the sperm concentration and progressive motility along with increased sperm abnormalities (Table 2). Moreover, the activities of testicular enzymes such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and gamma glutamyl transferase were increased, while the activity of acid phosphatase was decreased (Table 3).

Marshal decreased the serum level of T_3 and T_4, while serum TSH concentration and relative weight of thyroid gland were increased (Table 4).

Regarding serum biochemical parameters, blood glucose level and ALT , AST activities were decreased at both doses and the level of total cholesterol was increased, while total protein and albumin were not changed (Table 5).

Histopathological examination of testes showed degeneration of leydig cells as well as some of the spermatogenic cells in low dose and severe degeneration of the spermatogenic cells along with interstitial edema in high dose (Fig. 1). Also, the livers were congested with focal areas of degeneration and necrosis in low dose (Fig. 2) and extensive proliferation of bile ducts, and

Table (1): Effect of marshal on body weight and relative weight of sexual organs of male rats afte roral administration for 65 successive days (n=5).

Group	Dose (mg/kg b.wt)	Body weight (g)	Relative weight of sex organs			
			Testes	Seminal vesicles	Prostate glands	Epididymis
Control (saline)	0.00	168.00 ±5.70	1.16 ±0.07	0.67 ±0.05	0.41 ±0.03	0.46 ±0.02
Marshal (1/20 of LD_{50})	4.20	158.00 ±8.34	1.50* ±0.08	0.58 ±0.06	0.18** ±0.02	0.33** ±0.01
Marshal (1/50 of LD_{50})	10.50	153.00 ±7.91	1.50 ±0.07	0.59 ±0.05	0.59 ±0.02	0.25** ±0.06

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Table (2): Changes in epididymal sperm characters of rats after 65 days administration of marshal (n = 5).

Group	Dose (mg/kg b.wt)	Epididymal sperm characters		
		Concentration (x 10^6/ml)	Progressive motility (%)	Abnormalities (%)
Control (saline)	0.00	316.00 ±1.40	75.00 ±1.58	1.80 ±0.01
Marshal (1/20 of LD_{50})	4.20	139.0** ±3.16	61.00** ±3.15	4.12** ±0.19
Marshal (1/50 of LD_{50})	10.50	64.0** ±0.60	30.00** ±2.11	9.30** ±0.27

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Table (3): Effect of marshal on some testicular enzymatic activities after oral administration for 65 successive days (n = 5).

Group	Dose (mg/kg b.wt)	Testicular enzymatic activity			
		Acid phosphatase (U/gm testis)	Alkaline phosphatase (IU/gm testis)	Lactate dehydrogenase (IU/gm testis)	Gamma glutamyl transferase (U/gm testis)
Control (saline)	0.00	1.76 ±0.04	5.30 ±0.14	4.16 ±0.14	0.10 ±0.004
Marshal (1/20 of LD_{50})	4.20	1.20** ±0.04	7.35** ±0.27	6.08** ±0.21	0.13** ±0.003
Marshal (1/50 of LD_{50})	10.50	0.92** ±0.06	8.05** ±0.37	6.83** ±0.18	0.15** ±0.003

** Significant at $P < 0.01$.

Table (4) : Effect of marshal on relative thyroid weight and some thyroid parameters after oral administration for 65 days (n = 5).

Group	Dose (mg/kg b.wt)	Relative weight of thyroid glands	TSH (µU/ml)	T_3 (ng/dl)	T_4 (µg/dl)
Control (saline)	0.00	0.12 ±0.003	2.44 ±0.11	82.80 ±1.21	3.76 ±0.20
Marshal (1/20 of LD_{50})	4.20	0.13* ±0.002	3.02* ±0.15	70.40** ±0.93	2.68** ±0.25
Marshal (1/50 of LD_{50})	10.50	0.14** ±0.002	3.22** ±0.16	63.80** ±1.02	2.02** ±0.13

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Table (5): Effect of marshal on some plasma biochemical parameters after oral administration for 65 successive days (n = 5).

Group	Dose (mg/kg b.wt)	Clucose (mg/dl)	Total cholesterol (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	ALT (U/ml)	AST (U/ml)
Control (saline)	0.00	118.05 ±9.6	52.9 ±0.90	7.87 ±0.28	3.15 ±0.09	158.42 ±2.9	105.24 ±0.97
Marshal (1/20 of LD_{50})	4.20	45.61** ±0.002	62.1** ±1.20	7.11 ±0.25	3.25 ±0.16	75.60** ±1.58	45.56** ±1.81
Marshal (1/50 of LD_{50})	10.50	48.46** ±2.57	0.14** ±1.00	7.61** ±0.30	3.18 ±0.22	70.76** ±2.26	29.70** ±3.30

** Significant at $P < 0.01$.

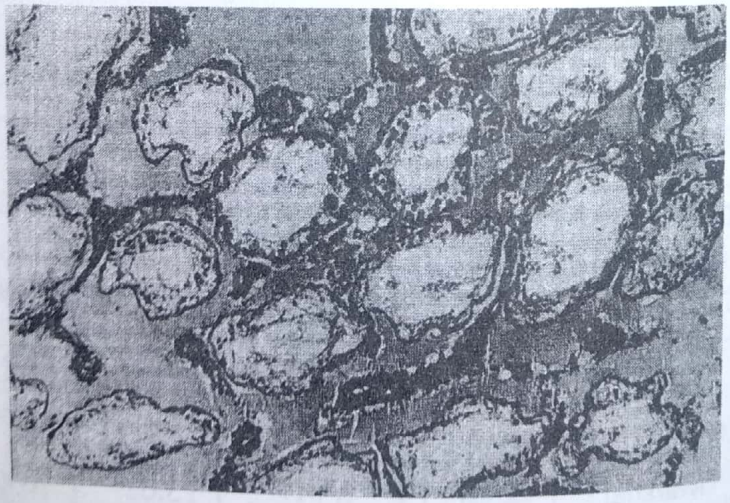

Fig. (1): Effect of marshal (10.5 mg/kg b.wt.)on testis of a rat. The cross section showed interstitial edema and degeneration of some spermatogenic cells (H & E , X400).

Fig. (2):Cross section of rat liver (4.20 mg/kg b.wt.) revealed congestion and periportal mononuclear cells infiltration (H & E , X250).

fibrosis at the portal areas as well as individual necrosis of some hepatocytes at high dose.

DISCUSSION

Marshal increased the weight of testes and decreased the weight of prostate glands and epididymes. These results are supported with that obtained by other structurally similar carbamates; Thiram (Mishra et al., 1993), Carbofuran (Pant et al., 1995A) and Mancozeb (Kackar et al., 1997A). The increased weight of testis may be explained on the basis of the interstital edema in the histopathology of the present study. The epididymal sperm characters including sperm concentration and motility were Signficantly reduced, while sperm abnormalities showed significant increase at both doses of marshal. The results assured by that of Pant et al (1996) after Carbaryl treatment and Pant et al (1995A) and Pant et al (1997) after Carbofuran treatment. In addition, Carbendazim (methyl 2-benzimidazole carbamate) has been reported to disrupt the microtubules of Sertoli cells (Nakai et al., 1995) and induce rapid direct effects on meiotic spermatocytes and latent effects on spermatids, leading to morphological abnormalities and failure of spermiogenesis in rats (Nakai and Hess, 1997).

Testicular enzymes associated with post-meiotic spermatogenic cells such as alkaline phosphatase and lactate dehydrogenase were increased while acid phosphatase was decreased after administration of marshal. In addition , the testicular enzymes associated with pre-meiotic spermatogenic cells or Sertoli cells such as gamma glutamyl transferase showed significant

increase. The results of testicular enzymes are supported by the epididymal sperm characters and the histopathology of the testis in the present study. Also, this findings are consistent with that of Mishera et al. (1993), , Pant et al. (1995A), Pant et al. (1995B), Kackar et al. (1997A) and Pant et al. (1997).

The lower dose of marshal induced degeneration of leydig cells as well as some of the spermatogenic cells. Also, the high dose caused the same histopathological changes with sever degeneration of the spermatogenic cells of some semineferous tubules with interstitial edema. A similar histopathological changes were observed in cross sections obtained from the testes of adult rats exposed to Carbendazim (Nakai et al., 1995), Carbofuran (Pant et al., 1995A and Pant et al., 1997), and Mancozeb (Kackar et al., 1997A).

Marshal decreased the serum level of T_3 and T_4 and increased TSH and the relative weight of thyroid gland . These results are consistent with that of Kackar et al (1997B), they found that Mancozeb (carbamate) caused an increase of thyroid/body weight ratio and reduced the thyroid iodine uptake, serum protein bound iodine, thyroxine (T_4) and the activity of thyroid peroxidase. These thyroidal changes may be explained on the basis that, carbamates (diethofenocarb) leads to an increase in hepatic UDP-glucuronyl transferase activity and acceleration of thyroxine excretion from the liver (Hosokawa et al., 1992). This acceleration causes a decrease in serum free T4 level, triggering the feedback mechanisms of the pituitary gland, promotion of TSH release and consequently an increase in serum TSH level.

Moreover, dithiocarbamate fungicides (Nabam) and its metabolite ethylene thiourea decrease serum level of T_3 and T_4 and induced ultrastructural changes namely increased number of myelin bodies, dilation of the rough endoplasmic reticulum, and increased vacuolization in the epithelial cells of the thyroid follicles (Kurttio et al., 1986).

Increased thyroid weight has been found to be associated with hypertrophy and hyperplasia of the follicular cells of thyroid gland of rats after Mancozeb treatment (Kackar et al., 1997B).This findings might explain the increased relative weight of thyroid gland in the present study.

Regarding serum biochemical parameters, blood glucose level and ALT and AST activities were decreased and total cholesterol was increased, while total protein and albumin were not changed after Marshal treatment in the present study. The results of blood glucose, total protein and albumin are consistent to that of Fayez and Kilgore (1992) after Oxamyl carbamate treatment, also they found that liver glucose-6-phosphatase was inhibited which explains the decreased blood glucose level. Moreover, Carbofuran acute toxicity did not affect blood total protein and albumin (Gupta et al., 1994).The increased total cholesterol are supported by the findings of Mishra et al. (1993) and Kackar et al. (1997A) after Thiram and Mancozeb treatment,

respectively.

Intracellular enzymes normally present in the plasma can be assumed to result from wear and tear of cells. This contribution of enzymes to the circulating blood may fall, either as the result of a genetic deficiency of enzyme production or depression of enzyme production as a result of disease (Moss et al., 1986). The activity of ALT and AST were reduced after oral administration of Marshal at both levels in the present study. Diethylthiocarbamates suppress the elevated plasma ALT activity induced by hepatotoxic substances and decrease microsomal drug-metabolizing enzyme activity in the liver (Masuda and Nakayama, 1982). Also, dithiocarbamate decreases the elevated AST activity after cis-diamminedichloroplatinum toxicity (Shimada et al., 1993). On the other hand, AST and ALT activities were elevated after subcutaneous injection of diethylcarbamate (Ishiyama et al., 1990).

Histopathological changes of rats received low dose of Marshal revealed slight congestion with focal areas of degeneration and necrosis of liver. In addition, the high dose showed extensive proliferation of the bile ducts and fibrosis at the portal areas as well as individual necrosis of some hepatocytes. These histopathological changes are assured by Ishiyama et al. (1990) after subcutaneous injection of diethylcarbamate.

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