

Effect of Carbofuran Pesticide on Male reproductive organs, Semen Analysis and Thyroid

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SUMMARY

The present study aimed to investigate the effect of Carbofuran pesticide on male reproductive organs, semen analysis and thyroid gland in rats. Oral administration of Carbofuran in doses of 4.2 and 10.5 mg/kg. B.Wt. to male rats daily for 65 successive days significantly increased the weight of testes and decreased the weight of prostates and epididymis. Epididymal sperm characters such as sperm motility and concentration were reduced, while sperm abnormalities were increased. The activity of testicular enzymes such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and gamma glutamyltransferase (γ -GT) were increased, while the activity of acid phosphatase was decreased. Carbofuran increased the weight of thyroid gland and plasma level of thyroid stimulating hormones (TSH), while triiodothyronine (T3) and thyroxine (T4) levels were reduced. Biochemical analysis revealed that Carbofuran decreased serum glucose level, AST and ALT enzymes, while total cholesterol was increased. Thus Carbofuran has been shown to induce marked functional changes in male reproductive organs and thyroid gland of rats.

Key words: Testes, prostates , epididymis, lactate dehydrogenase, gamma glutamyltransferase, alkaline phosphatase ,thyroid stimulating hormones, triiodothyronine, thyroxine.

INTRODUCTION

Carbamates are part of a large group of synthetic pesticides that have been developed in the last 40 years. Commonly used in agriculture as Carbamates are insecticides, herbicides, fungicides and nematodes 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 and fish (**Gupta, 1994**).

Carbofuran (2, 3-Dihydro-2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate) is a broad spectrum systemic carbamate pesticide that kills insects, mites and nematodes on contact and after ingestion (**Kamboj et al., 2008**). Carbofuran and other carbamate pesticides have widely replaced the more persistent and hazardous organophosphate pesticides used in agriculture due to its relatively short residual life in the environment and rapid degradation (**Eisler, 1985**). Owing to this, Carbofuran was reported to leach into the ground and surface water thus carrying the risk to various consumers and organisms (**Farahani et al., 2008**). Carbofuran acts by

inhibiting the acetylcholinesterase enzyme but unlike the organophosphate pesticides, the activity is reversible (**Baron, 1991**). It was also reported to cause reduction in epididymal sperm count and to decrease the libido in male rabbits and rats (**Pant *et al.*, 1995 A and Baligar and Kaliwal, 2004**).

It is well known that the thyroid gland plays 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 (**Kackar *et al.*, 1997B and Hosokawa *et al.*, 2002**). Therefore, the present study was performed to verify the effect of Carbofuran on reproductive system, thyroid gland and some biochemical parameters in male rats.

MATERIAL AND METHOD

Materials:

I-Carbofuran: (2, 3-Dihydro-2, 2-dimethyl-7-benzofuranyl-methylcarbamate) was obtained from Central Agricultural pesticide Laboratory,

II-Animals: Sixty mature male rats were used. Rats were fed on ordinary ration and water was provided *ad libitum*.

Experimental Design:

Fifteen mature male albino rats (150-170 gm) were divided into three groups, five rats each. The first group was kept as a control. The second and third groups were given Carbofuran orally in doses of 4.2 and 10.5 mg/kg.B.Wt. daily for 65 successive days respectively (**Hall *et al.*,1990**).

After 65 days, the rats were weighted and blood sample was obtained from each rat, left to clot and the serum was separated for biochemical analysis and plasma were separated for hormonal studies. Then these rats were sacrificed for studying the effect of Carbofuran male reproductive system and thyroid gland.

A-Effect on Reproductive System

Thirty mature male rats (150-180g) were divided into 3 groups. The first group was kept as a control, whereas the second and third groups were administered orally profenofos in doses of 0.36 and 3.6 mg/kg.b.wt. which is equal to 1/100 and 1/10 of LD50 respectively daily for 65 successive days to cover a complete spermatogenic cycle (**Hershberger *et al.*, 1969**). The sexual organs weight and the epididymal sperm characters were determined according to **Bearden and Fluquary, (1980)**. One testis from each animal was homogenized according to **Hodgan and Sherins (1973)** for determination of the following testicular enzymes; acid phosphatase according to (**Babson and Read, 1959**), alkaline phosphatase (**Roy, 1970**), lactate dehydrogenase (**Buhl and Jackson, 1978**) and gamma glutamyltransferase (**Szasz, 1969**).

B- Effect on Thyroid Gland:

The thyroid glands were removed and weighted individually for calculation of their relative weights. Plasma levels of T3 and T4 (**Britton et al, 1975**) and TSH (**Jackson, 1982**) were measured.

C- Biochemical Studies:

They were collected from each rat at the end of the experiment. Blood samples were taken from retro-orbital venous plexus of veins into clean, sterile and labeled centrifuge tubes to separate serum to determine some biochemical parameters as follows Serum aspartate aminotransferase (AST) and alanine transferase (ALT) were determined according to **Reitman and Frankel (1957)**, Total cholesterol (T. chol.) was estimated according to the method described by **Watson (1960)**. Glucose was measured spectrophotometrically according to the method described by **Trinder, (1969)**. Total protein (TP) and albumin (ALb) were determined according to method described by **Weichselbaum (1946)** and **Doumas et al., (1971)**, respectively.

D-Statistical analysis:

The results were subsequently analyzed following the statistical methods established by **Snedecor and Cochran (1980)** in order to determine whether a dose group was positive or negative.

RESULTS AND DISCUSSION

Carbofuran increased the weight of testes and decreased the weight of prostates and epididymis (**Table 1**). These results are supported with that obtained by **Pant et al., (1995)**; **Ferdinand et al., (2007)** and **Siti et al., (2012)**. The epididymal sperm characters including sperm concentration and motility were reduced, while sperm abnormalities were increased at both doses of tested insecticide (**Table 1**). These results were assured by **Pant et al., (1997)**; **Ferdinand et al., (2007)**; **Siti et al., (2012)** and **Kaur et al., 2015**. **Nakai et al., (1995)** has been reported that Carbofuran disrupts the microtubules of Sertoli cells and induces rapid direct effects on meiotic spermatocytes and latent effects on spermatids, leading to morphological abnormalities and failure of spermatogenesis in rats (**Nakai and Hess, 1997**).

Testicular enzymes associated with post- meiotic spermatogenic cells such as ALP and LDH were increased while acid phosphatase was decreased after administration of tested insecticide (**Table 2**). In addition, the testicular enzymes associated with pre-meiotic spermatogenic cells or Sertoli cells such as γ -GT enzyme was increased. The results of testicular enzymes are supported by the epididymal sperm characters in the present study. These findings are consistent with those of **Kacker et al.,(1997A)**; **Pant et al., (1997)**; **Siti et al., (2012)** and **Kuppusamy and Kadarkari (2017)**.

Carbofuran decreased the plasma level of T3 and T4 while increased TSH and relative weight of thyroid gland (**Table 3**). These results are consistent with those of **Kacker et al, (1997B) and Slotkin et al., (2013)**. These authors found that carbamate caused an increase of thyroid/ body weight and reduced the thyroid iodine uptake, serum protein bound iodine, T4 and the activity of thyroid peroxidase. These thyroidal changes may be explained on the basis that carbamates lead to acceleration of thyroxine excretion from liver (**Hosokawa et al., 2002**). This acceleration causes a decrease in serum free T4 level, promotion of TSH release and consequently an increase in serum TSH level.

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 (**Kacker et al., 1997B**). This findings might explain the increased relative weight of thyroid gland in the present study.

Regarding serum biochemical parameters, glucose level, ALT and AST were decreased and total cholesterol was increased, while total protein and albumin were not changed (**Table 4**). The results of glucose level, total protein and albumin are consistent to those of **Fayez and Kilgore, (2005) and Ferdinand et al., (2007)**. After carbamate treatment, also they found that liver glucose-6- phosphatase was inhibited which explains the decreased blood glucose level. The increased total cholesterol are supported by the findings of **Kacker et al., (1997A) and Ferdinand et al., (2007)**.

The activity of ALT and AST enzymes were reduced after oral administration of the tested insecticide at both dosage levels in the present study. Diethylcarbamates suppress the elevated plasma ALT activity induced by hepatotoxic substances and decrease microsomal enzyme activity in the liver (**Masuda and Nakama, 2002**). Also, diethylcarbamates activity decreased the elevated AST after carbamates toxicity (**Shimada et al., 2009**).

CONCLUSION:

It could be concluded that Carbofuran pesticide produces adverse effects on male reproductive organs and semen picture as well as on thyroid gland function. Therefore a great attention should be taken during the use of Carbofuran in male animals.

Table (1): Effect of Carbofuran on fertility in male rats after oral administration for 65 successive days (n=5).

Group	Dose mg/kg BWt	Relative Weight of sexual organs (gm/100g.B.Wt.)				Epididymal sperm characters		
		Testis	S.V.	Prostate	Epididy.	Conc. (10 ⁶ /ml)	Motility %	Abnormal ities %
Control	-----	1.16 ±0.07	0.67 ±0.05	0.41 ±0.03	0.46 ±0.02	316 ±1.4	75 ±1.58	1.8 ±0.01
Carbofuran	4.2	1.50* ±0.08	0.58 ±0.06	0.18** ±0.02	0.33** ±0.01	139** ±3.16	61** ±3.15	4.12** ±0.19
	10.5	1.50 ±0.07	0.59 ±0.05	0.59 ±0.02	0.25** ±0.06	64** ±0.60	30** ±2.11	9.3** ±0.27

S.V. = Seminal vesicles

Values represent means ± SE at * P < 0.05 ** P < 0.01

Table (2):- Effect of Carbofuran on some testicular enzymatic activities after oral administration for 65 successive days (n=5).

Group	Dose mg/kg B.Wt.	Testicular enzymes activities			
		Acid phosphatase (U/g testis)	ALP (IU/g testis)	LDH (IU/g testis)	γ-GT (U/g testis)
Control	-----	1.76±0.04	5.3±0.14	4.16±0.14	0.10±0.004
Carbofuran	4.2	1.2**±0.04	7.35**±0.27	6.08**±0.21	0.13**±0.003
	10.5	0.92**±0.06	8.05**±0.37	6.83**±0.18	0.15**±0.003

Values represent means ± SE at ** P < 0.01

Table (3):- Effect of Carbofuran on relative thyroid weight and thyroid weight and hormones after oral administration for 65 successive days (n=5).

Group	Dose mg/kg. B.Wt.	Relative weight of thyroid glands	TSH (μ U/ml)	T ₃ (ng /dl)	T ₄ (ng /dl)
Control	-----	0.12 \pm 0.003	2.44 \pm 0.11	82.8 \pm 1.21	3.76 \pm 0.2
Carbofuran	4.2	0.13* \pm 0.002	3.02* \pm 0.15	70.4** \pm 0.93	2.68** \pm 0.25
	10.5	0.14** \pm 0.002	3.22** \pm 0.16	63.08** \pm 1.02	2.02** \pm 0.13

Values represent means \pm SE at * P < 0.05 ** P < 0.01

Table (4):- Effect of Carbofuran on some biochemical parameters after oral administration for 65 successive days (n=5)

Group	Dose mg/kg. B.Wt.	Glucose (mg/dl)	T.Chol. (mg/dl)	T. Prot. (g/dl)	Albumin (g/dl)	ALT (U/ml)	AST (U/ml)
Control	----- -	118.05 \pm 9.6	52.9 \pm 0.9	7.87 \pm 0.28	3.15 \pm 0.09	158.42 \pm 2.9	105.24 \pm 0.97
Carbo- furan	4.2	45.61** \pm 0.002	62.1** \pm 1.2	7.11 \pm 0.25	3.25 \pm 0.16	75.6** \pm 1.58	45.56** \pm 1.81
	10.5	48.46** \pm 2.57	0.14** \pm 1.0	7.61 \pm 0.3	3.18 \pm 0.22	70.76** \pm 2.26	29.7** \pm 3.3

Values represent means \pm SE at ** P < 0.01

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