Acute Toxicological Evaluation of Low Dose Methyl 2-benzimidazole Carbamate Fungicide on Male Albino Rats

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ABSTRACT

Background: Methyl 2-benzimidazole carbamate (carbendazim) is a synthetic fungicide used against organisms that cause different types of plant diseases. It is widely used as a preservative in leather, paint, textile, fruit, and paper industry. It is also used as an anticancer drug in clinical medicine.

Methods: To evaluate the low dose toxicity of this compound in mammalian tissues, carbendazim was administered to male albino rats at 5, 10, 25, and 50 mM doses intradermally. At 6, 12 and 24 hours, blood samples were collected from the animals for analysis of biochemical and haematological parameters including serum enzyme activities.

Results: The findings indicated that carbendazim caused a decrease in serum total protein , ALT, AST, ALP and amylase activities. GGT activity decreased by 6hr and increased by 12hr in a dose-related manner. Carbendazim caused an increase in serum cholesterol, uric acid, glucose, and creatinine content while serum phosphorous content decreased. Mean hemoglobin, WBC, E, and platelet counts increased and total RBC, N and L counts decreased. Tissues (brain, heart, liver, kidney, and testis) taken from 50mM dose administered rat for histopathological examination revealed liquefactive necrosis of blood vessels, fibrosis, dialated parenchyma, hemorrhage, and edema in the brain tissue. In heart, necrosis and loss of nuclei in cardiac muscle cells were noted.

Conclusion: Low dose level of carbendazim causes toxicological effects in the rat tissues.

Keywords: Carbendazim, Enzymes, Eistopathology, Protein, Tissues

INTRODUCTION

Pollutants in the environment cause various hazards directly in living species. Most environmental pollutants are chemical pesticides. Carbendazim, a broad spectrum benzimidazole carbamate fungicide with systemic activity, is used against various degrees of fungal diseases in field crops, fruits, ornamental plants, and vegetables. On the other hand, carbendazim at higher doses (50mM) has been noted to have toxic effects on a variety of experimental animals. The fungicidal properties of carbendazim have been identified to be due to the binding of carbendazim to tubulins, an effect that disrupts microtubule formation and mitosis (1).

Carbendazim has been reported to have an antimitotic effect on human granulosa cells by interfering with microtubule and centrosome organization during mitosis (2). Dietary administration of carbendazim for up to 90 days produced minimal effects on the liver weight in female rats exposed to 360 mg/kg per day (3). Carbendazim induced hematological, biochemical, and histopathological changes to liver and kidneys of male rats when administered orally at 0, 150, 300, and 600 mg/kg/day for 15 weeks (4). Yet, carbendazim is widely used to prevent and control plant diseases caused by fungi. The present study was, therefore, designed to investigate the low dose toxicological profile

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of carbendazim in the brain, heart, liver, kidney, and testicular tissues of male rats.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (180-200mg weighing) were obtained from the animal house JIPMER, Puducherry. The rats were divided into five groups of six each. One group was taken as the control study and the other four as treatment groups. All animals were kept under controlled conditions of temperature (22±10°C) and humidity (60±5°). They were given pellet food and drinking water ad libitum. A 12 hour day and night cycle was maintained in the animal house.

Test substance

Carbendazim (>96% pure) technical grade was obtained from Gharda Chemicals Ltd, Mumbai, India.

Experimental design

This study was performed according to good laboratory practices. Carbendazim dissolved in ethanol was administered (5, 10, 25, and 50mM) intradermally to different groups of male rats for tissues, blood, and serum quantitative determination at 6, 12, and 24hr durations. All rats were given diethyl ether as an anesthetic. Approximately 3 ml of blood was collected from the heart of male albino rats for hematological and clinical and biochemical evaluation. An additional 1ml of blood was collected into EDTA coated tubes for determining hemoglobin (HB) content, erythrocyte (RBC), total leucocyte (WBC), differential leucocytes (N, E, L), and platelet counts. Two milliliters of the collected blood was processed to obtain serum, by immediately keeping the sample on dry ice and centrifuging it at 5000 rpm for 10 minutes, and used subsequently for the quantitative determination of total protein (TP) by Lowry (5), enzyme activities of alanine and aspartate aminotransferases (ALT and AST) by Reitman and Frankel (6), alkaline phosphatase (ALP) by Bessey (7), gamma glutamyltranspeptidase (GGT) by Volohonsky (7) and amylase by Bernfeld (8) methods. Likewise, quantitative determination of cholesterol (CHOL) by Zaks (9), urea (UR) by Natelson (10), uric acid

(UA) by Caraway (11), glucose (GLU) by Asatoorand King (12), albumin (AL) by Reinhold (13), creatinine (CREAT) by Owen (14), calcium (CA) by Gitelman (15) and phosphorous (PHOS) by Goldenberg's (16) methods was conducted. The organs (brain, heart, liver, kidneys, and testis) selected for the study were maintained in neutral buffered formalin (10%). Tissues for histological examination were processed, embedded in paraffin, sectioned at 5µm thickness, and stained with hematoxylin/eosin. Histological examination was performed on the tissues from control and 50mM dose groups.

Statistical analysis

The analysis of variance test (ANOVA) was used to evaluate the hematological and biochemical data for each measurement taken, and statistical significance ($P<0.05$ and $p<0.01$) was followed by a comparison of the group using Dunnett's test.

RESULTS

Enzyme specific activities

The results for serum enzyme specific activities of control male rats and carbendazim (5, 10, 25, and 50mM/kg bwt) treated rats determined after 6, 12 and 24 durations are shown in Table1. ALT specific activity significantly decreased ($P<0.01$) at all doses of carbendazim and durations except for the 5 and 50mM doses for which the ALT specific activity increased at 24hr time point. Tissue AST specific activity significantly increased for 5 and 50mM doses at 6hr and also for the 25mM dose at 12hr, whereas at other doses and durations, it ($P<0.01$) decreased significantly. ALP specific activity increased significantly at 6hr for the 5, 25 and 50mM doses and at 24hr duration for the 10, 25, and 50mM ($P<0.01$) doses. At 12hr, ALP specific activity in response to all the doses significantly ($P<0.01$) decreased in the serum of rats. Serum GGT specific activity increased at 6hr for 5, 10 and 25mM doses, while it decreased significantly for the 50mM dose in rat serum. Similarly, at 24hr, the 25mM dose level only augmented the GGT specific activity, whereas other doses significantly reduced ($P<0.01$) the GGT specific activity. At

12hr, the effect of various doses of carbendazim increased significantly (P<0.01) GGT specific activity as noted. Amylase specific activity decreased significantly (P<0.01) at 6 and 12hr, and at 24hr, amylase specific activity in response to 5 and 50mM carbendazim decreased significantly (P<0.01) in rat serum.

Biochemical studies

Serum (Table-2) total protein content decreased significantly (P<0.01) at 6, 12 and 24hr for all doses of carbendazim, while for the 25mM dose at 6hr, it increased and no significant changes in protein was noted for the 25mM and 50mM doses. Cholesterol level increased significantly (P<0.01) in a dose-related manner at 24hr duration. Urea level decreased significantly by 10mM dose at 12hr and by 10mM and 50mM doses at 24hr. The level of uric acid increased significantly (P<0.01) at 6, 12 and 24hr in a dose-related manner. Serum glucose level increased significantly (P<0.01) by the low dose of 5mM and the high dose of 50mM at 6hr, by 50mM at 12hr and by all doses at 24hr. The doses of 10mM and 25mM at 6hr, and 5, 10, and 25mM at 12hr significantly decreased the serum glucose level in the rats. Albumin content was significantly reduced by the low dose of 5mM but increased for 10mM dose at 6hr. The 25mM dose significantly decreased albumin at 12hr. Similarly, at 24hr, 10, 25, and 50mM doses decreased significantly, whereas the 5mM dose significantly (P<0.01) increased the serum albumin. Creatinine level in rat serum generally increased significantly for all doses and at all durations, except for the high dose at 6hr and 25mM dose at 12hr. Calcium level in rat serum at 6hr showed increases at all doses of carbendazim. Although significant decreases were seen for 5, 10, and 50mM doses at 12 and 24hr, the 25mM dose at 24hr showed a significant increase. Phosphorous level increased significantly at the low dose of 5mM at 12 and at 24hr and at the high dose of 50mM at 6 and 24hr.

Hematology

The results of the hematological studies are shown in Table 3. The mean concentration of hemoglobin significantly increased at 6, 12,

and 24hr time points. For all doses and at all durations, RBC mean values and mean WBC count increased significantly (P<0.01). At 24hr, Neutrophil count changed. Mean eosinophil count increased (P<0.01) at 6 and 12hr, whereas at 24hr it decreased (P<0.05) for the low (5mM) and high doses (50mM). Lymphocyte count decreased (P<0.01) at 6 and 24hr, whereas it increased significantly for the low dose (5mM) at 24hr, but it did not show a significant change at 12hr. Platelet count changed at all durations such as at 6hr for 10mM dose, 12hr for 10, 25, and 50mM, and for all doses at 24hr. However, platelet count in rat blood decreased at 6hr for 25 and 50mM doses and at 12hr for the 5mM dose.

Histopathology

Carbendazim caused histopathological changes in the brain, heart, liver, kidneys, and testis of male rats in the 50mM dose group at 6, 12, and 24hr post intradermal administrations of the compound. In rat brain (Figure1), liquefactive necrosis of blood vessels at 6hr, fibrosis and dialated parenchyma and hemorrhage at 12hr, and dialated blood vessels and necrosis with hemorrhage and edema at 24hr were observed. In rats heart (Figure 2), necrosis with edema and hemorrhage at 6hr, necrosis and loss of nuclei at striations at 12hr, and waviness of fibres and coagulation necrosis at 24hr were noted post treatment. In rats liver (Figure 3), the central vein dialated with increased sinusoids at 6hr, congested blood vessels and central vein, and proliferation of macrophages near central vein at 12hr, and mild fatty liver changes with sinusoid dialation at 24hr were noted compared to control liver tissue. In kidneys (Figure 4), congestion of convoluted tubules with thickened bowman's capsule at 6hr, hypercellular glomeruli with polymorph infiltration and mild necrotic changes at 12hr, and abortive changes of tubules and glomeruli with thickening of bowman's capsule at 24hr were noted. In testis (Figure 5), mild hypertrophy of the cells of seminiferous tubules (sclerosis) at 12 and at 24hr was noted compared to the control tissue.

Fig 1: **Brain** histopathological findings by 50mM carbendazim (CAR) dose group in male rats

CONTROL   CAR -6hr

Normal Architecture   Liquefactive necrosis of blood vessels

CAR -12hr   CAR -12hr

Fibrosis and dialated parenchyma & hemorrhage   Dialated blood vessels and necrosis with hemorrhage and edema
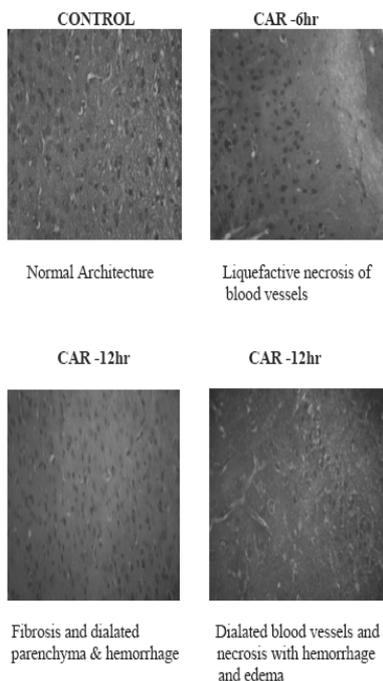
Fig 2: **Heart** histopathological findings by 50mM carbendazim (CAR) dose group in male rats

CONTROL   CAR -6hr

Normal Architecture   Necrosis with edema and hemorrhage

CAR -12hr   CAR -12hr

Necrosis and loss of nuclei and striations   Waviness of fibers and coagulation necrosis
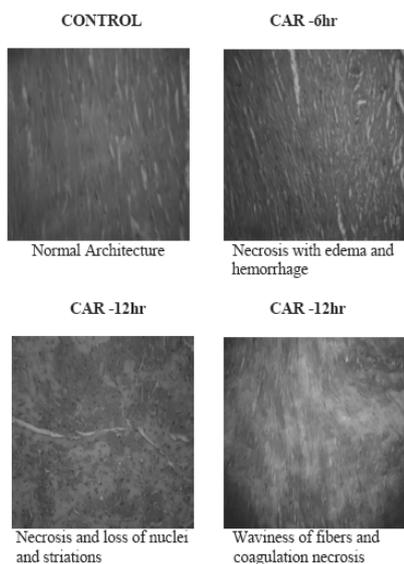
Fig 3: **Liver** histopathological findings by 50mM carbendazim (CAR) dose group in male rats

CONTROL   CAR -6hr

Normal Architecture   Central vein dialated with increased sinusoids

CAR -12hr   CAR -12hr

Congested blood vessels and central vein, proliferation of macrophages near central vein   Mild fatty liver changes with sinusoidal dialation
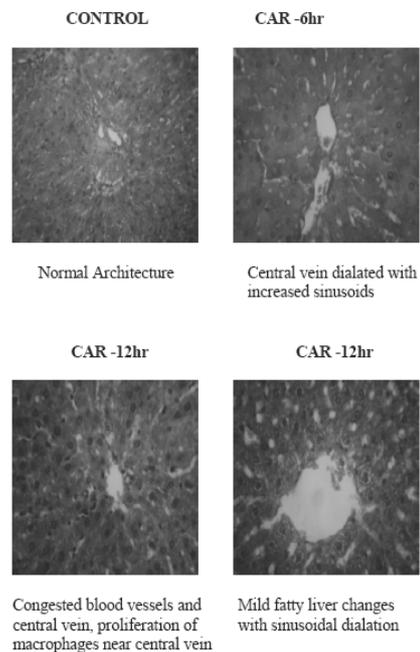
Fig 4: **Kidney** histopathological findings by 50mM carbendazim (CAR) dose group in male rats

CONTROL   CAR -6hr

Normal Architecture   Congestion of convoluted tubules with thickened Bowman's capsule

CAR -12hr   CAR -12hr

Hypercellular glomeruli with polymorphs infiltration and mild necrotic changes   Abortive changes of tubules glomeruli with thickening of Bowman's capsule
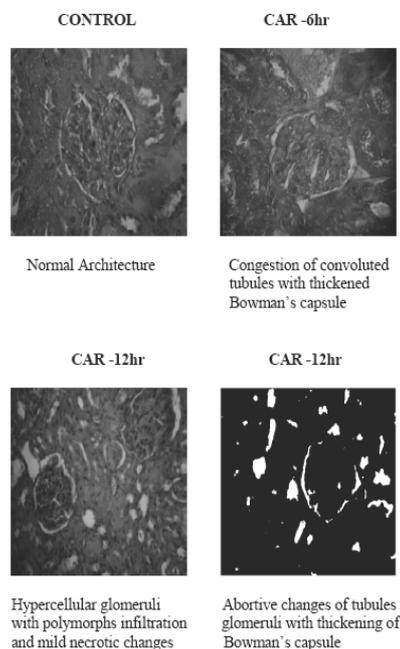
Fig 5: **Testis** histopathological findings by 50mM carbendazim (CAR) dose group in male rats

CONTROL

Normal Architecture

CAR -6hr

Mild hypertrophy of the cells of seminiferous tubule

CAR -12hr

Hypertrophy of seminiferous tubules and its cells (sclerosis)

CAR -12hr

Sclerosis of seminiferous tubules
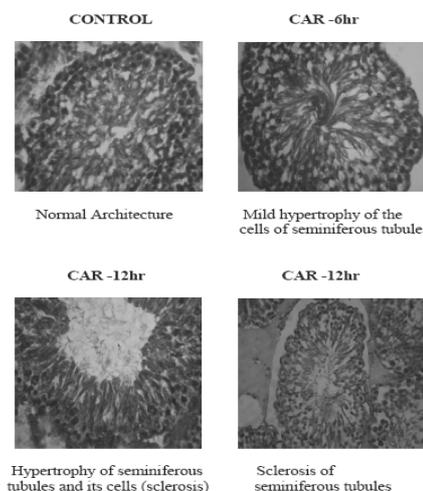
Table 1: Results of enzyme specific activity of rats in control and carbendazim (CAR) treated groups.

Parameter	Duration	Control	5mM CAR	10mM CAR	25mM CAR	50mM CAR
ALT (IU/mg protein/ml x10⁻²)	6hr	0.153 ± 0.017	$0.079 \pm 0.009^{**}$	$0.088 \pm 0.008^{**}$	$0.116 \pm 0.007^{**}$	$0.137 \pm 0.017^{**}$
	12hr	0.351 ± 0.027	$0.281 \pm 0.017^{**}$	$0.301 \pm 0.021^{**}$	$0.343 \pm 0.023^{**}$	$0.307 \pm 0.006^{**}$
	24hr	0.163 ± 0.011	$0.169 \pm 0.013^{**}$	$0.159 \pm 0.01^{**}$	$0.162 \pm 0.014^{**}$	$0.179 \pm 0.012^{**}$
AST (IU/mg protein/ml x 10⁻²)	6hr	0.337 ± 0.027	$0.505 \pm 0.030^{**}$	$0.190 \pm 0.027^{**}$	$0.244 \pm 0.025^{**}$	$0.375 \pm 0.034^{**}$
	12hr	0.520 ± 0.073	$0.238 \pm 0.028^{**}$	$0.485 \pm 0.026^{**}$	$0.594 \pm 0.040^{**}$	$0.471 \pm 0.037^{**}$
	24hr	0.349 ± 0.020	$0.325 \pm 0.024^{**}$	$0.232 \pm 0.029^{**}$	$0.226 \pm 0.022^{**}$	$0.260 \pm 0.021^{**}$
ALP (IU/mg protein/ml x 10⁻²)	6hr	0.545 ± 0.060	$0.557 \pm 0.034^{**}$	$0.508 \pm 0.037^{**}$	$0.711 \pm 0.039^{**}$	$0.790 \pm 0.036^{**}$
	12hr	0.829 ± 0.040	$0.561 \pm 0.033^{**}$	$0.600 \pm 0.031^{**}$	$0.609 \pm 0.036^{**}$	$0.792 \pm 0.037^{**}$
	24hr	0.605 ± 0.046	$0.597 \pm 0.028^{*}$	$0.660 \pm 0.038^{**}$	$0.680 \pm 0.046^{**}$	$0.770 \pm 0.052^{**}$
GGT (IU/mg protein/ml x 10⁻²)	6hr	0.014 ± 0.0004	$0.01 \pm 0.0007^{**}$	$0.011 \pm 0.0007^{**}$	$0.011 \pm 0.0012^{**}$	$0.019 \pm 0.0008^{**}$
	12hr	0.015 ± 0.0007	$0.016 \pm 0.0005^{**}$	$0.024 \pm 0.0005^{**}$	$0.017 \pm 0.0006^{**}$	$0.019 \pm 0.0008^{**}$
	24hr	0.013 ± 0.0006	$0.01 \pm 0.0005^{**}$	$0.011 \pm 0.0007^{**}$	$0.014 \pm 0.0007^{**}$	$0.008 \pm 0.0006^{**}$
Amylase (IU/mg protein/ml x 10⁻²)	6hr	1.16 ± 0.077	$0.701 \pm 0.065^{**}$	$0.998 \pm 0.062^{**}$	$1.07 \pm 0.089^{**}$	$1.05 \pm 0.072^{**}$
	12hr	1.89 ± 0.101	$1.63 \pm 0.142^{**}$	$1.77 \pm 0.114^{**}$	$1.83 \pm 0.113^{**}$	$1.76 \pm 0.135^{**}$
	24hr	1.19 ± 0.188	1.16 ± 0.114	$1.35 \pm 0.110^{**}$	$1.36 \pm 0.118^{**}$	$0.951 \pm 0.091^{**}$

Values are mean ± SEM from 6 rats in each group. Statistically significant at $p \leq 0.05$ = * and $p \leq 0.01$ = **.

Table 2: Results of biochemical analysis of rats in control and carbendazim (CAR) treated groups.

Parameter	Duration	Control	5mM CAR	10mM CAR	25mM CAR	50mM CAR
Protein (g/dL)	6hr	6.10 ± 0.191	5.92 ± 0.273**	6.21 ± 0.100**	6.10 ± 0.129	6.10 ± 0.106
	12hr	6.10 ± 0.161	5.50 ± 0.209**	5.25 ± 0.174**	5.10 ± 0.250**	5.20 ± 0.193**
	24hr	6.21 ± 0.086	6.00 ± 0.093**	5.90 ± 0.081**	5.70 ± 0.077**	5.60 ± 0.112**
Cholesterol (mg %)	6hr	43.66 ± 2.37	44.00 ± 1.18	46.00 ± 1.15	46.00 ± 1.06	47.00 ± 2.01
	12hr	45.00 ± 2.55	39.00 ± 1.69	43.00 ± 2.29	48.00 ± 1.87	49.00 ± 1.57
	24hr	43.00 ± 1.31	62.00 ± 2.59**	74.00 ± 2.17**	75.00 ± 2.97**	77.00 ± 2.12**
Urea (mg %)	6hr	38.0 ± 2.64	45.0 ± 4.54	40.0 ± 2.88	46.0 ± 4.28	50.0 ± 3.43
	12hr	48.0 ± 3.95	46.5 ± 4.01	38.0 ± 2.47**	43.0 ± 2.58	46.0 ± 4.06
	24hr	28.0 ± 2.67	25.0 ± 1.82	15.0 ± 1.09**	20.0 ± 1.00	16.0 ± 0.894**
Uric acid (mg/dL)	6hr	12.4 ± 0.165	14.5 ± 0.159**	14.8 ± 0.230**	15.0 ± 0.134**	16.0 ± 0.335**
	12hr	12.7 ± 0.193	13.1 ± 0.143**	14.0 ± 0.214**	14.4 ± 0.256**	15.0 ± 0.278**
	24hr	11.0 ± 0.157	13.6 ± 0.188**	14.0 ± 0.428**	14.3 ± 0.332**	14.5 ± 0.348**
Glucose (mg/ml)	6hr	1.20 ± 0.103	1.67 ± 0.060**	0.99 ± 0.073**	0.77 ± 0.044**	2.54 ± 0.085**
	12hr	1.3 ± 0.082	1.09 ± 0.06**	1.19 ± 0.053**	1.22 ± 0.042**	2.45 ± 0.093**
	24hr	0.6 ± 0.038	0.95 ± 0.055**	0.98 ± 0.072**	1.35 ± 0.057**	0.868 ± 0.058**
Albumin (gm %)	6hr	3.06 ± 0.152	2.70 ± 0.211**	3.60 ± 0.173**	3.10 ± 0.201	3.15 ± 0.180
	12hr	3.20 ± 0.173	3.30 ± 0.180	3.20 ± 0.198	2.90 ± 0.274**	3.20 ± 0.198
	24hr	3.50 ± 0.222	4.00 ± 0.219**	3.20 ± 0.169**	2.90 ± 0.203**	3.10 ± 0.268**
Creatinine (mg /dL)	6hr	0.3 ± 0.044	0.33 ± 0.051**	0.31 ± 0.044**	0.2 ± 0.036**	0.3 ± 0.044
	12hr	0.4 ± 0.044	0.41 ± 0.057**	0.5 ± 0.057**	0.4 ± 0.068	0.51 ± 0.057**
	24hr	0.5 ± 0.068	0.51 ± 0.068*	0.56 ± 0.057**	0.6 ± 0.081**	0.7 ± 0.077**
Calcium (mg/dL)	6hr	10.0 ± 0.421	10.5 ± 0.413**	11.0 ± 0.339**	10.8 ± 0.345**	10.9 ± 0.340**
	12hr	11.0 ± 0.461	8.90 ± 0.348**	10.4 ± 0.383**	11.0 ± 0.338	9.90 ± 0.358**
	24hr	11.1 ± 0.340	9.60 ± 0.392**	9.90 ± 0.330**	13.8 ± 0.349**	8.71 ± 0.415**
Phosphorous (mg/dL)	6hr	7.00 ± 0.536	7.00 ± 0.296	8.10 ± 0.270**	7.00 ± 0.300	8.60 ± 0.413**
	12hr	6.00 ± 0.264	6.50 ± 0.316**	5.93 ± 0.359	6.80 ± 0.251**	6.20 ± 0.371
	24hr	6.00 ± 0.115	7.00 ± 0.262**	6.30 ± 0.194**	6.00 ± 0.269	7.60 ± 0.318**

Values are mean ± SEM from 6 rats in each group. Statistically significant at p≤0.05 = * and p≤0.01 = **.

Table 3; Results of haematological analysis of rats in control and carbendazim (CAR) treated groups.

Parameter	Duration	Control	5mM CAR	10mM CAR	25mM CAR	50mM CAR
HB (gm/dL)	6hr	11.3 ± 0.73	12.9 ± 0.45	15 ± 0.34**	12.9 ± 0.28	12.9 ± 0.57
	12hr	12.3 ± 0.60	15.6 ± 0.37**	13.4 ± 0.38	15.6 ± 0.36**	12.5 ± 0.26
	24hr	12 ± 0.72	12.5 ± 0.38	12.5 ± 0.51	14.6 ± 0.78*	12.5 ± 0.58
RBC (mill / cu.mm)	6hr	2.6 ± 0.146	2.1 ± 0.123*	2.5 ± 0.131	2.2 ± 0.106	2.4 ± 0.093
	12hr	4.15 ± 0.211	3.2 ± 0.129**	2.5 ± 0.157**	3.4 ± 0.121*	2.4 ± 0.106**
	24hr	4.8 ± 0.233	2.3 ± 0.152**	2.1 ± 0.251**	4.6 ± 0.288	2.8 ± 0.229**
WBC (x 10⁹/cu.mm)	6hr	5.25 ± 0.271	5.8 ± 0.146**	6.7 ± 0.107**	8.2 ± 0.146**	5.5 ± 0.100**
	12hr	6.2 ± 0.313	8.27 ± 0.108**	9.4 ± 0.173**	9.9 ± 0.151**	9.0 ± 0.111**
	24hr	5.4 ± 0.196	9.4 ± 0.124**	8.1 ± 0.159**	7.9 ± 0.111**	7.4 ± 0.147**
N (%)	6hr	63 ± 1.06	59 ± 1.77	67 ± 1.73	68 ± 2.35	70 ± 1.57
	12hr	64 ± 1.80	63 ± 2.20	67 ± 1.87	66 ± 2.88	65 ± 1.94
	24hr	61 ± 1.82	45 ± 2.16**	70 ± 1.59	71 ± 1.69*	67 ± 2.22
E (%)	6hr	2 ± 0.258	2 ± 0.577	3 ± 0.447**	3 ± 0.258**	3.5 ± 0.577**
	12hr	4 ± 0.577	4 ± 0.577	5 ± 0.577**	5 ± 0.447**	4 ± 0.577
	24hr	5 ± 0.577	4 ± 0.577*	4.5 ± 0.516	5 ± 0.577	3 ± 0.577**
L (%)	6hr	35 ± 0.577	37 ± 0.930	30 ± 1.29**	28 ± 0.930**	27 ± 1.15**
	12hr	32 ± 1.77	35 ± 2.11	32 ± 1.06	27 ± 0.93	29 ± 1.39
	24hr	31 ± 0.577	49 ± 1.06**	26 ± 1.23**	20 ± 1.00**	30 ± 1.15
Platelet (lakhs/cu.mm)	6hr	3.3 ± 0.166	3.3 ± 0.141	3.5 ± 0.169**	3.1 ± 0.121**	3.2 ± 0.100**
	12hr	3.3 ± 0.129	3.1 ± 0.159**	3.6 ± 0.216**	3.5 ± 0.191**	3.5 ± 0.159**
	24hr	2.8 ± 0.177	3.2 ± 0.123**	3.8 ± 0.123**	4.1 ± 0.180**	5.6 ± 0.148**

Values are mean ± SEM from 6 rats in each group. Statistically significant at p≤0.05 = * and p≤0.01= **.

DISCUSSION

Individuals get exposed to carbendazim through their occupation and/or through food consumption. Primary exposure for the general human population will be from residues of benomyl and carbendazim used in food crops. Very limited research related to the effects of carbendazim on tissues (such as brain, heart, liver, kidney and testis) had been done earlier on mammals. The present study, therefore, investigated the acute effect of carbendazim on male rat tissues from biochemical, hematological, and histopathological points of view. In this study, significant changes in serum enzymes activities were noted that suggested liver toxicity. Both induction and inhibition of enzyme activities were observed in the present study. ALT and AST enzyme activities decreased significantly in rats treated with carbendazim. Since benomyl was metabolized to carbendazim, it was reported that benomyl administration orally or intraperitonially (500mg) reduced the enzyme activity of hepatic microsomal mixed function oxidases in the rats (17). Serum ALP level in contrast increased at short and long durations (18) following carbendazim treatment of rats. GGT catalyzed the transfer of gamma-glutamyl group to a wide variety of amino acid acceptors (19). GGT was localized to the focal areas of hepatocytes (20). GGT was found to increase in preneoplastic lesions of the liver during chemical carcinogenesis (21). Abnormally high levels of GGT were also observed in tumors of a variety of tissues, including hepatocellular carcinomas (22, 23). Shukla reported that increases in the serum and liver GGT levels of rats were indicative of a toxic or preneoplastic response of the liver to benomyl(24). In the present study, a significant change in the serum GGT level of male rats treated with carbendazim was noted that may ,therefore, be indicative of a carbendazim induced carcinogenic potential developing in rats. The inhibition of amylase activity, on the other hand, reflected decreased carbohydrate metabolism in the tissues as a result of carbendazim toxicity. Significant decreases in serum total protein and albumin were noted in the male rats. The decrease in protein content could be due to a decrease in the rate of protein synthesis. Rats fed with 50, 150, 450 and 1350ppm carbendazim in the diet for 13 weeks yielded urine and blood chemistry within the normal range. Female rats that received 1350ppm carbendazim, however, exhibited a reduction in total protein content (WHO / IPCS 1993). In a different study, carbendazim administered daily to rats at 0, 150, 300 and 600mg/kg/day by gavage for 15 weeks decreased the serum total protein content at lower dose levels (4). Increases in the amount of albumin were noted at 6 and 24hr durations. Increase in the amount of albumin may, therefore, be explained by the carbendazim treatment of rats. Albumin content synthesized by the liver most often transports or binds drugs or chemicals (4). On the contrary, cholesterol level increased in the serum due to liver and kidney damage. An elevated amount of serum cholesterol was observed in dogs fed with 500mg carbendazim/kg for 1 year or longer (25). Similarly, carbendazim administered orally to male rats (Rattus rattus) for 15 weeks caused an increase in albumin, glucose, creatinine, and cholesterol levels (4). Serum glucose levels increased significantly at 50mM dose of carbendazim administered to rats and for all durations. The increase in glucose level may be attributed to the disruption of glucose intake and use by cells (3). The decrease in urea content may be due to decreased amino acid metabolism as a result of carbendazim toxicity (4). The amount of uric acid, however, was noted to be higher along with increased serum creatinine level in rats fed with carbendazim. It is reported that increase in creatinine content generally occurs with renal failure (26). Serum calcium content increased at 6hr and decreased at 12 and 24hr, while the phosphorous level remained elevated in the serum. As for the hematological analysis, carbendazim caused an increase in hemoglobin content, white blood cells, eosinophil count, and platelet count in a dose related manner, but it decreased red blood cell, lymphocyte, and neutrophil counts. The decrease in red blood cells may indicate a

disruption of erythropoiesis or an increase in destruction of red blood cells (27). The latter is more probable since increase in hemoglobin content is noted alongside the decrease in RBC counts. Similarly, it was reported that carbendazim caused a dose dependent decrease in RBC and lymphocyte numbers in rats (4). The increase in the WBC count indicated enhanced immune capacity and increase in eosinophil numbers suggested eosinophilia. Histopathological findings observed through microscopic investigations were related to the high dose of carbendazim affecting the brain, heart, liver, kidneys, and testis of male rats. We observed the necrosis of blood vessels in the brain, heart, and liver. Enlargement of sinusoid, vacuolation of hepatocyte cytoplasm, and congestion of blood vessels and central vein in the liver were noted in the treated rats. In the treated kidneys, changes in thickening of Bowman's capsule and tubules were observed. Similarly in testis, hypertrophy and sclerosis of seminiferous tubules were observed. In previous studies, similar results had been observed during oral treatment with benomyl (28) or its metabolite, carbendazim (18, 27). The current data suggests that at low doses, carbendazim elicited toxic effects in the various organs of rat through affecting biochemical and hematological parameters resulting in histopathological changes. The use of this pesticide in countries where pesticides are widely used without regulation may cause health hazards to non target organisms at various levels, including those to human beings.

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