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Biochemical evaluation of low dose methyl 2-benzimidazole carbamate fungicide on male albino rats

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Abstract

Methyl 2-benzimidazole carbamate (carbendazim) is one of the synthetic fungicides that controlled organisms that caused plant diseases of different types. It is widely used as a preservative in leather, paint, textile, fruits and papermaking industry. It is also used as an anticancer drug in chemical medicine. In the present study low concentrations of carbendazim was administered at 5, 10, 25 and 50mM doses intradermally to male albino rats. At the end of 6 hr, 12hr and 24hr duration, blood samples were collected from the animal for the analysis of biochemical and haematological parameters. Carbendazim caused an increase of cholesterol, uric acid, glucose and creatinine while serum phosphorous content was decreased. However, mean hemoglobin, WBC, E, and platelet counts increased and total RBC, N and L counts decreased. These results indicated that low dose level carbendazim contributed to toxicological effects in the rat tissues.

Keywords: Methyl 2-benzimidazole carbamate; Fungicide; Rat tissues

Introduction

Pollutants in the environment cause various hazards directly on 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 disease in field crops, fruits, ornamental plants and vegetables. On the other hand, carbendazim is noted to have toxic effects on a variety of experimental animals. The fungicidal properties of carbendazim has been identified as due to the binding of carbendazim to tubulins, an effect that distrupted microtubule formation and mitosis [1]. It was reported that in human granulosa cells, carbendazim had an antimitotic effect by interfering with microtubule and centrosome organization during mitosis [2]. Dietary administration of carbendazim for upto 90 days produced minimal effects on the liver weight in female rats exposed to 360mg/kg per day [3]. Carbendazim induced haematological, biochemical and histopathological changes to liver and kidney of male rats when administered orally at 0, 150, 300 and 600 mg/kg/ day for 15 weeks [4]. Yet, carbendazim was used widely to prevent and control plant diseases caused by fungi. The present study was therefore designed to investigate the low dose toxicological profile of carbendazim in the brain, heart, liver, kidney and testicular tissues of male rat.

Materials and methods

Animals

Male albino wistar rats (180-200gm weighing) were obtained from the animal house JIPMER, Puducherry. Rats were divided into five groups, each containing six animals. One of the groups was control and the

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other four were treatment groups. All the 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 twelve 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.

Experimental design

This study was four performed according to good laboratory practices. Rats were divided into a control and test groups, each group containing six animals. Carbendazim dissolved in ethanol was administered intradermally (5mM, 10mM, 25mM and 50mM) to the different groups at 6hr, 12hr and 24hr duration. All rats were given diethyl ether as an anaesthetic. Approximately 3 ml of blood was collected for hematological and clinical and biochemical evaluation. An additional one milliliter of blood was collected into EDTA coated tubes determination of hemoglobin (HB) content, erythrocyte (RBC), total leucocyte (WBC), differential leucocytes (N, E, L) and platelet counts.

Two milliliter of blood were processed to obtain serum that was immediately frozen on dry ice and centrifuged at 5000 rpm for 10 minutes, and used subsequently for the quantitative determination of the following was carried out for cholesterol by the method of Zaks *et al* [5], urea by Natelson *et al*, [6], uric acid by Caraway [7], glucose by Asatoor and King [8], albumin by Reinhold [9], creatinine by Owen *et al*, [10], calcium by Gitelman [11] and phosphorous by Goldenberg [12].

Statistical analysis

An 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.

Result

Biochemical studies

Serum (Table 1) total protein content significantly decreased ($p<0.01$) at 6hr, 12hr and 24hr for all doses of carbendazim, while for the 25mM dose at 6hr, it was increased and no significant changes in protein was noted for the 25mM and 50mM doses. Cholesterol level was significantly ($p<0.01$) increased in a dose related manner at 24hr duration. Urea level was significantly decreased by 10mM dose at 12hrs and by 10mM and 50mM doses at 24hrs. The level of uric acid increased statistically significant ($p<0.01$) at 6 hr, 12 hr and 24 hr in a dose related manner. Serum glucose level was significantly increased ($p<0.01$) by the low dose of 5mM and the high dose of 50mM at 6hr, by 50mM at 12hr and by all doses at 24 hr. The doses of 10mM and 25mM at 6hr, and 5, 10 and 25mM at 12 hr significantly decreased the serum glucose level in the rat. Albumin amount was decreased significantly by the low dose of 5mM but increased for 10mM dose at 6hr. The dose of 25mM significantly decreased albumin at 12hr. Similarly, at 24hr, the doses of 10mM, 25mM and 50mM significantly decreased, whereas at low 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 high dose at 6 hr and 25mM at 12hr. Calcium level in rat serum at 6 hr showed increase at all doses of carbendazim. Although, significant decrease was seen for 5mM, 10mM and 50mM dose at 12hr and 24 hr, the 25mM dose at 24 hr showed significant increase. Phosphorous level increased significantly at the low dose of 5mM at 12 hr and 24 hr time point and similarly at the high dose of 50mM showed statistically significant increase of serum phosphorous at 6 hr and 24 hr.

Hematology

Results of the hematological studies are shown in table 2. The mean concentration of hemoglobin significantly increased at 6hr, 12hr and 24hr. RBC mean values generally increased significantly. The mean WBC count increased significantly ($p<0.01$) for all doses and at all durations. Neutrophil count significantly changed at 24hr in the rat blood. Mean eosinophil count significantly ($p<0.01$) increased at 6hr and 12hr, whereas it decreased significantly ($p<0.05$) for the low (5mM) and high doses (50mM) at 24hr. Lymphocyte count decreased statistically ($p<0.01$) at 6hr and 24hr, whereas for the low dose (5mM) at 24hr increased significantly, but showed no significant change in lymphocyte count at 12 hr. Platelet count changed significantly at all durations

such as at 6hr for 10mM dose, at 12hr for 10mM, 25mM and 50mM, and for all doses at 24hr. Platelet counts in rat blood decreased however at 6hr for 25 & 50mM and at 12hr for the 5mM dose.

Table 1. 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 $p \leq 0.05$ = * and $p \leq 0.01$ = **.

Discussion

Individuals may get exposed to carbendazim through occupation and or consumption of food products. Primary exposure for general human population will be from residues of benomyl and carbendazim used on food crops. Very limited research related to the effects of carbendazim on tissues (such as brain, heart, liver, kidney and testis) as well as on biochemical and heamatological parameters had been done earlier on mammals. The present study, therefore investigate the acute effect of carbendazim on male rat tissues from a biochemical, hematological and histopathological points of view.

Decrease in serum total protein and albumin were noted with in male rats. Decrease in protein content could be due to a decrease in the rate of protein synthesis. Rats fed 50, 150, 450 and 1350ppm carbendazim in the diet for 13 weeks, yielded urine and blood chemistry within normal range. Female rats that received 1350ppm carbendazim however exhibited reduction of total protein content [3]. In another study, carbendazim administered daily to rats at 0, 150, 300 and 600mg/kg/day by gavage for 15 weeks, decreased the serum total protein at lower dose levels [4]. The increased amount of albumin showed at 6 and 24hr duration. Albumin content synthesized by the liver – most often transports or binds drugs or chemical. So the increase in the amount of albumin may be explained by carbendazim

treatment [4]. 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 [13]. Similarly, carbendazim administered orally to male rats (Rattus rattus) for the 15 weeks, caused an increase of albumin, glucose, creatinine and cholesterol levels [4].

Table 2. 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 10^3/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 $p \leq 0.05$ = * and $p \leq 0.01$= **.

Blood serum glucose levels increased statistically significant at 50mM doses of carbendazim treated rats at all duration. The increase of glucose level may be attributed to 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 was however noted to be alongwith increased, serum creatinine level in rats fed carbendazim. It is however known that increase of creatinine occured with renal failure [14]. Serum calcium content increased at 6hr, and decreased at 12 and 24 hr, while the phosphorous level was also noted to be increased in the serum.

As for the hematological analysis, carbendazim caused increase in hemoglobin content, white blood cells, eosinophil count and platelet count in a dose related manner but 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 cell. The latter in more probable since increase in hemoglobin content is noted alongside decrease in RBC counts. Similarly, it was reported that carbendazim caused a dose dependent decrease in RBC and lymphocyte values in rats [4]. Increase in the WBC count indicated enhanced immune capacity and indicated the eosinophilia by the increase in eosinophil numbers.

Conclusion

The invest data suggests that at low dose elicited toxic effects in the various organs of rat through affecting biochemical and hematological parameters resulting in histopathological changes. Use of this pesticide in countries where pesticides were widely used without regulation may cause health hazard at various levels to nontarget organisms, including that of human beings.

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