Toxic effects of carbendazim at low dose levels in male rats

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ABSTRACT — Carbendazim is a systemic broad-spectrum fungicide controlling a wide range of pathogens. It is also used as a preservative in paint, papermaking and leather industry, and as a preservative of fruits. In the present study, low dose intracellular effect of carbendazim was investigated employing 5, 10, 25 and 50 mM of the compound administered to male rats intradermally. Blood and liver of each animal was collected 6 hrs later to analyze serum and tissue enzyme activities, tissue lipid peroxidation and hematological and biochemical parameters. The experimental results of low dosage carbendazim use indicated augmentation of investigated parameters. However, the higher dosage of carbendazim use resulted in renormalization of investigated parameters to control levels or to values below control, providing a U-shaped hormesis type dose-response profile. Histopathological sections revealed portal vein congestion, mononuclear cell infiltration and hydropic degeneration of the liver tissue. These results indicated that carbendazim even at low dose exhibited toxicity, affected the liver and also caused specific changes in hematological and biochemical parameters in the rat.

Key words: Carbendazim; Rat liver; AST/ALT; LPO; Hormesis; Histology

INTRODUCTION

All over the world different types of pesticides are used for control of different types of damages caused by pests. Carbendazim (methyl-2-benzimidazole carbamate) is one of the broad spectrum fungicide currently widely used and it’s major metabolite benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate] is also used as a fungicide (Vettorazzi, 1976). This compound is used as a preservative in paint, textile, paper and leather industry as well as a preservative of fruits (Selmanoglu et al., 2001). Carbendazim is an inhibitor of microtubule synthesis and directly alters testicular function through germ cell depletion and altering Sertoli and Leydig cell functions (Gray et al., 1989). Carbendazim is well absorbed (80-85%) after oral exposure and is subsequently metabolized into many compounds within the organism. The main metabolites are 5-hydroxy-2-benzimidazole carbamate (5-HBC) and 5, 6-hydroxy-2-benzimidazole carbamate-N-oxides (5, 6-HOBC-N-oxides). Carbendazim and its metabolites are however poorly catabolized and are retained in tissues such as gonads, liver, adrenals, adipose, skin and other organs (WHO, 1993).

The fungicidal property of methyl -2-benzimidazole carbamate is targeted towards tubulins, causing disruption of microtubule formation and mitotic cell division (Burland and Gull,1984; Foster et al., 1987; Can and Albertini,1997). There is little information on the other effects of this compound such as effects on animal liver and metabolic functions. Carbendazim and its parent precursor fungicide, benomyl show minimal acute toxicity with an oral LD₅₀ in male rats > 15 g/kg (Seiler,1975). However, carbendazim and related benzimidazoles show marked reproductive toxicity in rodents (Cummings et al., 1990; Gray et al., 1990; Nakai et al.,1992; Perreault et al.,1992). In rats, carbendazim and benomyl have been known to induce testicular toxicity, resulting in sloughing of immature spermatids (Parvinen and Kormano,1974; Hess et al., 1991) and in the inhibition of microtubule assembly (Lim and Miller,1997). Further, carbendazim exhibits developmental toxicity by way of developmental abnormalities such as embryonic death and growth retar-
dation in rodents when administered to prenatal rats during pregnancy (Cummings et al., 1992). Administration of benomyl to pregnant rats produced craniocerebral and systemic malformations including cleft palate, hydrocephalus, and exencephaly in fetal rats (Ellis et al., 1987). Nevertheless, carbendazim is used widely to prevent and control plant diseases caused by various fungi. The present study was therefore designed to evaluate the toxic effects of metabolic aberration and morphological changes that accompanied carbendazim use at lower doses and at given interval of time.

MATERIALS AND METHODS

Carbendazim was obtained from Gharda Chemicals Ltd., Mumbai, India. All chemicals used in this study were of analytical grade. Glass distilled water was used for preparation of all reagents. Male albino Wistar strain (8-10 weeks old) rats were used in the present study. The animals were housed in propylene cages under controlled temperature and hygiene conditions with 12 hr of light and dark cycle throughout the experimental period. The animals were provided free access to drinking water ad libitum. Rats were grouped into six for control and test animals. Carbendazim was solubilized in corn oil and was freshly prepared prior to administration to the animals. The doses chosen were 5, 10, 25 and 50 mM and the compound was administered intradermally to the male rats. Control group of rats were administered an equivalent amount of corn oil as was used for the test animals. The dose of carbendazim was selected arbitrarily to determine the minimal dose for which tissue and serum responses could be observed with respect to selected markers. Ultrastructure and histopathological changes that occurred correspond to such doses and will indicate the toxicity potential of the compound for animal tissues when encountered as dietary contaminants. Following intradermal administration of material, rats were anesthetized using anesthetic ether at 6 hrs after dosing.

Hematology

Hematological parameters such as Red cell, WBC, and Neutrophil (Garg and Goyal, 2006) counts and the serum content of hemoglobin (Pari and Murugavel, 2005), glucose (Perrone et al., 2005), Cholesterol (Zak et al., 1954), and Creatinine were determined for control as well as carbendazim treated rats (Malla Reddy and Bashamohideen, 1989).

Serum and liver biochemistry

Blood was drawn from the heart of each anesthetized rat, and allowed to clot. The blood samples were then centrifuged at 1500 rpm for 10 min at room temperature and serum was separated and used for the estimation of aspartate and alanine transaminase activities (AST&ALT), by the method of Reitmann and Frankel (1957). Liver was surgically removed from each anesthetized rat and washed in cold 1.15% KCl and homogenized in the same solution employing a Potter-Elvehjem homogenizer to obtain a 10% (w/v) tissue homogenate. The homogenate was then centrifuged in a Hitachi refrigerated high speed centrifuge at 10,000 × g and at 4°C for 10 min. The clear supernatant obtained was used as the enzyme source for the measurement of liver AST and ALT activities, and for lipid peroxidation (Placer et al., 1996). Serum and liver sample protein content was estimated by the method of Lowry et al., (1951).

Histopathology

Tissues were processed by standard histopathological techniques. Liver sections were cut at 5-8 μ thickness with a rotary microtome at room temperature and were fixed in a 10% neutral phosphate-buffered formalin solution for histopathological analysis. Liver sections were stained with the Giemsa stain and periodic acid–Schiff methods and then counterstained using hematoxylin as described by Simoes and Schoning for light microscopic examination (1994). Liver histology was evaluated and histopathological changes were scored according to the criteria of Oakberg and Hess (1956, 1990). Tissue sections were observed under a microscope at a magnification of 100X.

Statistical analysis

All the values were expressed as mean ± SEM. Difference between control and treated groups was assessed by ANOVA. A value of p<0.05 was considered statistically significant.

RESULTS

Serum AST and ALT activities were surprisingly found diminished due to 5, 10, and 25 mM carbendazim use whereas 50 mM dose of the compound yielded similar to or greater than control level activity of these enzymes. Serum cholesterol and creatinine values were however found gradually raising with increasing dose of the compound from 5 -50 mM. Red blood cell count reduced 3-19% and WBC count decreased 18-35% below control as a consequence to the increase in dose of carbendazim administered to rats. Although lymphocyte count increased 20% as a result of 5 mM carbendazim use, they
Table 1. Effects of carbendazim on serum and liver in male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>5 mM</th>
<th>10 mM</th>
<th>25 mM</th>
<th>50 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>2.6 ± 0.146</td>
<td>2.1 ± 0.123*</td>
<td>2.5 ± 0.131</td>
<td>2.2 ± 0.106*</td>
<td>2.4 ± 0.93</td>
</tr>
<tr>
<td>WBC</td>
<td>8200 ± 146</td>
<td>6700 ± 107</td>
<td>5800 ± 119*</td>
<td>5500 ± 100***</td>
<td>5250 ± 145</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>35 ± 0.58</td>
<td>37 ± 0.93</td>
<td>30 ± 0.290*</td>
<td>28 ± 0.930***</td>
<td>27 ± 1.15***</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>63 ± 1.1</td>
<td>59 ± 1.8</td>
<td>67 ± 0.9*</td>
<td>68 ± 1**</td>
<td>70 ± 1.5</td>
</tr>
<tr>
<td>Hb</td>
<td>11.3 ± 0.730</td>
<td>12.9 ± 0.456</td>
<td>15 ± 0.341***</td>
<td>12.9 ± 0.289</td>
<td>12.9 ± 5.76</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
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<tr>
<td><strong>chemistry</strong></td>
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<tr>
<td>AST</td>
<td>83.33 ± 6.2</td>
<td>37 ± 1.2***</td>
<td>55.33 ± 3.7**</td>
<td>67.16 ± 3.2*</td>
<td>83.66 ± 5.5</td>
</tr>
<tr>
<td>ALT</td>
<td>174.4 ± 9.2</td>
<td>167 ± 6.2</td>
<td>123 ± 9**</td>
<td>149 ± 8.2</td>
<td>229 ± 11**</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>62.7 ± 11.3</td>
<td>65.2 ± 4.3</td>
<td>73.5 ± 11.3</td>
<td>79.0 ± 15.5</td>
<td>100.8 ± 9.1*</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.73 ± 0.04</td>
<td>0.73 ± 0.01</td>
<td>0.74 ± 0.05</td>
<td>0.79 ± 0.05</td>
<td>1.01 ± 0.9*</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
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</tr>
<tr>
<td><strong>chemistry</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MDA</td>
<td>0.95 ± 0.11</td>
<td>0.65 ± 0.01**</td>
<td>0.567 ± 0.08*</td>
<td>0.687 ± 0.02*</td>
<td>0.709 ± 0.01</td>
</tr>
<tr>
<td>AST</td>
<td>92.55 ± 6.9</td>
<td>96.5 ± 9.2</td>
<td>97.98 ± 11.2</td>
<td>93 ± 7.8</td>
<td>94 ± 12</td>
</tr>
<tr>
<td>ALT</td>
<td>62.11 ± 3.9</td>
<td>67 ± 5.2</td>
<td>71 ± 4.9</td>
<td>63.03 ± 4.1</td>
<td>65.65 ± 7</td>
</tr>
</tbody>
</table>

AST & ALT values are expressed in U/L, cholesterol and creatinine values are expressed in mg/dl, Hb is expressed in gm/dl, RBC and WBC values are indicated in cumm, MDA values is expressed in nmol/mg of protein.
returned to 22% below control value, when 50 mM of the compound was used. Neutrophil counts however increased marginally with increase in dose of carbendazim. Hemoglobin content essentially remained at 12.9 mg/dL, existing at 14% above control value for 5, 25 and 50 mM carbendazim dose. However, 10 mM dose of the compound elevated the hemoglobin content to 36% above the control.

Liver AST and ALT activities in contrast exhibited an increase over their respective control values. The increase in enzyme activity in relation to the use of 5, 10, 25 and 50 mM carbendazim was in the range 1-4% over ALT control, while for AST, the corresponding changes were in the range 1-14.5% over AST control, respectively. The ratio of AST/ALT activity however remained around 1.4 in all cases. A marked reduction was however observed in the level of liver tissue lipid peroxidation in rats exposed to carbendazim. The malondialdehyde content of liver was found to be least for 10 mM carbendazim use when compared to each of the other concentrations employed (Fig. 1).

Histologically, portal vein congestion, enlargement of the sinusoids, increase in the number of liver Kupffer cells and mononuclear cell infiltration in the liver, were observed as a consequence of carbendazim treatment (Fig. 2). Hydropic degeneration of hepatocytes was also observed in the liver of treated rats (Fig. 3).

**DISCUSSION**

Humans get exposed to carbendazim via consumption of food. Primary exposure for the general human population will be to residues of benomyl and carbendazim in food crops (WHO/IPCS, 1993). There seems to be limited research carried out on the effects of carbendazim and benomyl on the biochemical and haematological parameters in mammalian liver. It was therefore interesting to evaluate, the effect of low dose carbendazim on selective biochemical and haematological parameters related to male rat liver. There was significant change in the serum AST and ALT activities of rats treated with carbendazim, whereas no change was identified in the liver tissue. Carbendazim had no effect on the haematological constituents of rats when administered at 80, 400, 2000 and 10000 ppm in their diet for 93 consecutive days (WHO/JMPR, 1974). Nevertheless, Igbedioh and Akinyele reported that AST and ALT enzyme activities increased significantly in rats treated with 520, 560 and 600 mg/kg benomyl for 7 days (1996). The differences in the results observed may be attributed to the exposure time to the pesticide. It was reported that benomyl administration orally and intraperitoneally (500 mg/kg) reduced the enzyme activity of hepatic microsomal mixed-function oxidase in rats (Dalvi, 1992). We found that lipid peroxidation in the liver tissue was significantly lowered relative to control values.

Carbendazim caused an increase in level of cholesterol in rats at higher doses. An increase in cholesterol level is a
sign of liver damage (Igbedioh et al., 1996). Elevated serum cholesterol was observed in dogs fed a diet containing 500 mg carbendazim/kg for 1 year or longer (Oakberg, 1956). Red blood cell count decreased in rats treated with 5 and 25 mM carbendazim and was statistically significant. White blood cell and lymphocyte counts also decreased significantly at 10 mM or more. Neutrophil counts, however, increased significantly at 10, 25 and 50 mM dose of carbendazim. The decrease in the number of red blood cells may indicate a disruption of erythropoiesis or an increase in the destruction of red blood cells (Thibodeau and Patton, 1993). Acute intoxication due to carbendazim generally decreased RBC and hemoglobin content, as also reduced reticulocyte and thrombocyte numbers in the blood of rats (Hayes, 1994). The decrease in white blood cells and lymphocyte count suggests that carbendazim may posses an immune-suppressive potential in rats.

Carbendazim at 50 mM also caused histopathological changes in the liver of the male rats. Congestion, mononuclear cell infiltration, hydropic degeneration, enlargement of the sinusoids and an increase in the number of liver Kupffer cells in carbendazim-treated rats were noted. Rat liver underwent severe histopathological lesions when treated with a single bolus dose of Carbendazim. Carbendazim is known to particularly affect the hepatocytes and renal corpuscles. Oral administration of carbendazim at 300 and 600 mg/kg per/body wt/day for 15 weeks to male rats, affected liver tissue and caused changes in hematological and biochemical parameters (Vural et al., 1986). Carbendazim exposure experimentally or occupationally, led to a decrease in the microsomal oxidation potential (Lisovska et al., 2005). Liver hypertrophy, hepatic necrosis, anomalous mitosis, induction of neoplastic nodules were some of the observations noted in experimental rats (Selmanoglu et al., 2001). However, Gray et al., reported that 400 mg/kg carbendazim did not cause any histopathological damage the liver of rats dosed from weaning until 105 days of age (Gray et al., 1990). In contrast male mice fed 5000 mg/kg carbendazim in diet showed centrilobular hypertrophy, necrosis and swelling of liver cells (Simoes and Schoning, 1994). Igbedioh and Akinyele have observed necrotized cells and oedema in the liver of rats given benomyl (WHO/IPCS, 1993). The differences in the results may be due to the age of the animals and exposure time to these compounds.

Results of our study, indicate that carbendazim caused changes in biochemical and haematological parameters, and in histopathology, even at the lower doses, and the decreases or the decreasing trends in serum AST/ALT as well as in the hepatic MDA levels noted against 5, 10 and 25 mM carbendazim dose, exhibited a “U”-shaped dose-response curve, probably reflecting hormesis like effects.

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