ABSTRACT

Commercial formulations and technical grade carbamate insecticides; propoxur, carbaryl and carbofuran were tested for their toxicity on in vitro cultured hepatocytes, lymphocytes, spleenocytes, myeloma cell lines, horn cancer cells and squamous cell carcinoma. Each of these insecticides was inoculated at 200, 400 and 600 ppm for 24, 48 and 72 hours. However tumor cells were incubated only with technical grade carbamates at 400 ppm for 24 hours. The percentage of abnormal cells in the primary cell culture and estimation of proteins, lactic acid and lactate dehydrogenase in the culture supernatant as an index of cell damage were used as a measure of the toxicity of the insecticides. Squamous cell carcinoma cells were further subjected to the measurement of the viable cell number using neutral red and proteins in the cell monolayer using methylene blue, in an ELISA reader. Osmotic fragility of erythrocytes, following exposure to technical grade carbaryl was also studied in order to assess the degree of anaemia, resulting upon exposure to carbamates.

The results indicated that, among the three commercial preparations of carbamate insecticides used; only Baygon spray (baygon) exhibited statistically significant toxic effects, whereas Sevin (carbaryl) and Carbogran 3G (carbofuran) did not show any toxic effect. Among the three cell types used, hepatocytes, owing to their detoxifying ability did not experience much toxicity of even baygon spray. However lymphocytes and spleenocytes did get affected by baygon spray but not by Sevin or Carbogran 3G. Baygon spray had inhibitory effects on the protein synthesis at the initial concentrations of 200 and 400 ppm but at 600 ppm due to increased cellular destruction the protein levels in the supernatant showed an increase. The amount of protein in Sevin and Carbogran 3G inoculated culture supernatant did not differ from that of control. Lactic acid and lactate dehydrogenase exhibited the same trend wherein they were found high in Baygon spray culture supernatants but no difference in values between control and treated groups in Sevin or Carbogran 3G treated culture supernatants in all the three types of cells studies.

Hepatocytes resisted the toxic effects, even of the technical grade carbamates. However lymphocytes and spleenocytes did succumb to the technical grade chemicals. These insecticides not only inhibited the growth of tumor cells but they also caused varying degrees of cell deformity/destruction and cell death. In hepatocyte culture, the extent of cell deformity was just twice that of control when technical grade insecticides were used. There was a greater inhibitory effect on protein concentration in the culture supernatant, with carbaryl effecting to the maximum extent. Lactic acid and lactate dehydrogenase concentration did not tally with the degree of cell damage, indicating that the carbamates inhibit the
cellular metabolism. Lactic acid showed an increase from 1.87±0.50 in control to 4.57±0.97, 1.19±0.50 and 8.12±1.33 mg/dl in 600 ppm baygon, carbaryl and carbofuran treated groups respectively. LDH release in hepatocytes with technical grade carbamates was 69.93±4.24 (U/L) in control and 89.91±3.53, 34.13±2.46 and 72.43±2.97 in 600 ppm treatment with baygon, carbaryl and carbofuran respectively. Lymphocytes showed greater extent of cell damage as compared to hepatocytes with technical grade carbamate insecticides. The abnormal lymphocytes were 84.25±1.95% when technical grade carbaryl was inoculated in the culture. The secretion of proteins by lymphocytes into the culture media was decreased due to the presence of the insecticides, which was evident by the fact that even at 600 ppm level the excessive cell death did not release much of protein. Lactic acid and LDH levels also showed a fall in the concentration as compared to control, due to inhibition of cellular metabolism by carbamates.

Spleenocytes with technical grade carbamates had abnormal cells in carbaryl treated group at 76.50±2.19 % more than the control. Protein (gm %), lactic acid (mg/dl) and LDH (U/L) recorded at 600 ppm with carbaryl (control) were 33.50±2.06 (10.00±0.71), 12.69±1.42 (21.32±2.33) and 20.81±1.38 (28.81±1.38) respectively.

Among the three types of tumor cells studied, horn cancer cells were more resistant to the technical grade insecticides, followed by myeloma cell lines and finally the most effected cell types were squamous cell carcinoma cells. It was observed that there was inhibition in the formation of monolayer or cell growth and multiplication. The abnormal cells with 400 ppm carbaryl at 24 hours were 49.75±3.90 for squamous cell carcinoma as compared to 8.50±0.56 for control. Myeloma cells and horn cancer cells showed 45.25±3.23% (11.25±1.98 in control) and 36.75±3.75 % (11.75±1.29 in control). The protein, lactic acid and LDH were significantly decreased in the treated groups (especially carbaryl treated groups) indicating inhibition in cellular metabolism.

The percent hemolysis of sheep erythrocytes without carbamates, initiated at 0.45% NaCl which reached 50% at 0.40 percent NaCl concentration. Complete hemolysis was seen at 0.35 percent NaCl. Baygon treated erythrocytes showed initial, 50% and complete hemolysis at 0.55, 0.45 and 0.35 % NaCl respectively i.e. a little earlier as compared to the control. Carbaryl treated erythrocytes showed initial, 50% and complete hemolysis at 0.65% NaCl, 0.55 % and 0.45 % concentration of NaCl respectively Carbofuran exhibited less toxic effects on the fragility of erythrocytes as compared to baygon and carbaryl, where in the initial, 50% and complete haemolysis was seen at 0.50, 0.45 and 0.40 percent NaCl respectively.