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Effects of Aldicarb and Malathion on Lipid Peroxidation

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Pesticides are the chemicals used for the controlling of the spread/reproduction of unwanted organisms, e.g. insects, weeds, fungi and rodents (Brander 1982, Matsumura 1985, Roberson and Nolan 1988).

Exposure to pesticides has been associated with many hazardous effects, including: acute and chronic toxicosis in human and animals, liver and heart diseases, hormonal disorders, skin diseases, mutagenic and carcinogenic events and effects on lipid peroxidation. For many years peroxidation in this context was overlooked but during recent decades interest in lipid peroxidation in living tissue has received serious attention as a potential toxicological hazard (Bagchi et al. 1995, Murphy 1980 Thines and Haley 1972, Yarsan 1998).

Several pesticides have been shown to stimulate peroxidation of cellular membranes (Dikshith 1991) including: organophophorus insecticides [nitroaromatic complex (parathion, methyl-parathion, paraoxon), vinyl-derivatives (phosphamidon, mevinphos, temivenphos), O,Odimethylmalathion, malathion, trichlorfon, sumithion], organo-halogenated pesticides [halogenated alkanes (carbon tetrachloride), tetrachlorodibenzo-dioxin, pentachlorophenol, hexachlorobenzene, dichlorodiphenyltrichloroethane, polychlorinated biphenyls, lindane, monuron, diuron], and herbicides (aminotriazole, paraquat). Pesticides and related compounds may contribute to the process of membrane peroxidation, by several mechanisms (Datta et al. 1994, Dikshith 1991, Gupta et al. 1992, Palmeirra et al. 1995, Parashar and Singh 1987) a. Direct initiation by free radicals produced by metabolism of the chemical. In this way, CCl_3 derived from CCl can initiate a chain of peroxidation by abstracting a hydrogen from other molecules. b. Indirect initiation by the production of reactive forms of oxygen during their metabolism. For example, paraquat can activate O_2 by univalent reduction to the superoxide anion (O_2). c. Inhibition of enzymatic systems of defence involved in the control of reactive oxidising entities. As an example, certain derived compounds of dithiocarbamates behave as inhibitors of Cu-Zn superoxide dismutase (Cu-Zn SOD). d. Destruction of natural antioxidants, which control the reactions of peroxidation. For instance, the reduction of the hepatocyte glutathione level induced by a fumigant (1,2-dibromoethane) is associated with the accumulation of malondialdehyde (MDA).

This study was undertaken to evaluate the effects on lipid peroxidation of aldicarb and malathion, which are widely used carbamate and organophophorus insecticides, respectively. This evaluation was done by determining Cu-Zn SOD, glutathione peroxidase

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(GSH-Px), and catalase activities in erythrocytes and MDA levels in plasma. Thus, these parameters are used as indicators for lipid peroxidation.

MATERIALS AND METHODS

Swiss albino mice (25-30 gram) were assigned into eight groups, consisting of fifteen mice each.

Mice of Group 1 were used as control for aldicarb. They were fed a commercial ration containing no pesticides. Mice of Group 2, 3 and 4 were given aldicarb orally (0.3; 0,6 and 1,2 mg/kg body weight, respectively). Mice of Group 5 were used as control for malathion and fed the commercial ration containing no pesticides. Mice of Group 6, 7 and 8 were given malathion orally (100; 500 and 1500 mg/kg/day, respectively).

Aldicarb [(2-methyl-2-(methylthio) propionaldehyde 0-(methylcarbamoyl) oxime], and malathion [(O,Odimethyl-S-(1,2-carbethoxyethyl) phosphorodithioate] were both of technical grade (95 %) and were purchased from Midiltipi Agricultural Drugs Corp. Ankara, Turkey. Pesticides were given continuously in the feed for the duration of the experiment (120 days). Aldicarb and malathion levels in feed were determined by thin layer chromatographic method (Ceylan 1980, Mills 1959) before given to mice. Tap water was freely available throughout the experiments.

Heparinized blood samples were collected from 5 mice by cardiac puncture on day 15, 45 and 120, representing subacute, subchronic and chronic exposure. Samples were centrifuged immediately (3.000 rpm, 15 min, 4 °C). The plasma and erythrocytes were separated by centrifugation, and these samples were stored at -70 °C until enzyme assays were performed.

Malondialdehyde levels in plasma were determined spectrophotometrically by reaction with 2-thiobarbituric acid (TBA) as described from Yoshioka et al. (1979). Cu-Zn superoxide dismutase activity in erythrocytes was measured by the previously detailed method of Fitzgerald et al. (1992). Glutathione peroxidase activity in erythrocytes was measured spectrophotometrically as described by Pleban et al. (1982). Catalase activity in erythrocytes was measured by using the spectrophotometric technique of Luck (1965).

Data were analysed statistically by one way analysis of variance (ANOVA). When significant treatment effects were detected, DUNCAN'S multiple range test was used to identify specific differences between treatment means at a probability level of 5%.

RESULTS AND DISCUSSION

Malondialdehyde content, GSH-Px, Cu-Zn SOD and catalase activities were determined in plasma or erythrocytes collected from the mice treated with aldicarb and malathion, on subacute, subchronic and chronic assays.

Studies on the effects of pesticides on lipid peroxidation are limited. Dowla et al., (1996) investigated, in vitro, the effects of acephate, cadmium, methamidophos, maleic hydrazide and nicotine on the erythrocyte enzyme activities such as delta-aminolevulinic acid dehydratase, Cu-Zn SOD, and plasma cholinesterase. They observed that methamidophos and other chemicals inhibited the plasma Cu-Zn SOD activities. Pedrajas et al. (1995)

studied the effects of xenobiotics, paraquat, $CuCl_2$, dieldrin and malathion on oxidative process. In that study, malathion enhanced significantly the microsomal TBARS (soluble thiobarbitüric acid reactive substances) while microsomal glutathione transferase activity decreased. The specific superoxide dismutase activity increased in animal exposed to Cu (II) but diminished in fish exposed to paraquat. Gromov et al. (1993) studied influence of the pesticides deltametrin and dichlorphos on memory processes, and the activity of antioxidant enzymes as Cu-Zn SOD and catalase in brain and blood of female rats. The enzyme activities were not significantly changed in blood. But catalase activity was lowered in brain (deltamethrin by 25%, dichlorvos 21%) 3 hours after their administration to these animals.

The above studies indicated that, pesticides stimulated lipid peroxidation of cellular membranes. Lipid peroxidation is a degenerative process, which effects the polyunsaturated fatty acids of membrane phospholipids. The general mechanism of this process involves the formation of toxic aldehydes, which react with protein and non-protein substances resulting in widespread changes in cellular membranes. These degenerative processes can be prevented by molecular and enzymatic ways. Molecular substances are vitamin A, vitamin C, vitamin E, uric acid, ceruleoplasmin; enzymes are Cu-Zn SOD, GSH-Px and catalase. Therefore, a lipid peroxidation event can be determined directly by the determination of reactive oxygen species (hydroxyl, superoxide anion, hydrogen peroxide and singlet oxygen radicals) or MDA levels. This event may be measured indirectly by the determination of molecular or enzymatical substances inhibiting lipid peroxidation (Comporti 1993, Dikshith 1991, Draper 1990, Yarsan 1998).

In the present study, aldicarb and malathion reduced Cu-Zn SOD (Table 1 and Table 2) and GSH-Px (Table 3 and Table 4) activities in erythrocytes. Important differences in Cu-Zn SOD activities were observed on subacute, subchronic and chronic periods in control and aldicarb treatment groups. The results of aldicarb treated groups were significantly different from those of control, and higher doses of aldicarb resulted in significantly increased Cu-Zn SOD activity.

Concerning the subacute exposure period, Cu-Zn SOD activities of group 7 and 8 were substantially different from malathion control group and group 6 (Table 2). In subchronic and chronic periods, malathion treated groups, among themselves, were not significantly different, but the results in these groups were significantly different from malathion control groups in these periods.

Table 1. Erythrocytes superoxide dismutase activities in aldicarb treated groups (U/gHb).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	783.00±34.02 *a*	778.00±42.51 a	754.60±55.25 a
	(725.00 - 810.00)	(705.00 - 810.00)	(663.00 – 800.00)
0.3 mg/kg b.w.	664.20±18.86 b	668.60±37.81 b	655.00±74.28 b
	(635.00 - 680.00)	(635.00 - 730.00)	(535.00 – 728.00)
0.6 mg/kg b.w.	605.00±17.80 c	617.60±80.12 b	626.60±67.67 b
	(580.00 - 630.00)	(485.00 - 686.00)	(520.00 – 705.00)
1.2 mg/kg b.w.	600.20±15.41 c	612.00±79.58 b	616.00±59.96 b
	(585.00 - 625.00)	(476.00 - 686.00)	(520.00 – 676.00)

a,b,c. Means within the same columns with different letters are statistically significant ($p<0.05$).

Table 2. Erythrocytes super-oxide dismutase activities in malathion treated groups (U/gHb).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	784.00±12.94 a	777.00±20.79 a	780.00±17.67 a
	(765.00 - 800.00)	(750.00 - 805.00)	(750.00 – 795.00)
100 mg/kg/day	686.80±24.66 b	567.20±74.85 b	553.40±18.78 b
	(658.00 - 725.00)	(489.00 - 688.00)	(530.00 – 580.00)
500 mg/kg/day	575.80±7.46 c	531.40±4.15 b	524.00±18.78 b
	(565.00 - 585.00)	(526.00 - 536.00)	(435.00 - 630.00)
1500 mg/kg/day	554.00±84.95 c	515.00±7.44 b	511.40±83.84 b
	(450.00 - 658.00)	(505.00 - 526.00)	(425.00 - 650.00)

a,b,c. Means within the same columns with different letters are statistically significant $p<0.05$).

No significant differences in GSH-Px activities were shown in the aldicarb treated groups on subacute period. However, important differences were observed when mice were exposed to aldicarb for 45 or 120 days.

In malathion treated groups, GSH-Px activities were found to be statistically different from those in malathion control group in the three experimental assays (subacute, subchronic and chronic). But significant differences were not found among the treatment groups.

Table 3. Erythrocytes glutathione peroxidase activities in aldicarb treated groups (U/gHb).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	39.20±2.68	39.20±1.30 a	42.00±5.83 a
	(35.00 – 42.00)	(38.00 - 41.00)	(35.00 - 49.00)
0.3 mg/kg b.w.	38.20±1.48	35.20±4.32 ab	33.60±1.14 b
	(36.00 – 40.00)	(31.00 - 42.00)	(32.00 - 35.00)
0.6 mg/kg b.w.	37.40±3.64	33.40±3.20 b	32.20±3.11 b
	(32.00 – 42.00)	(29.00 - 38.00)	(29.00 - 36.00)
1.2 mg/kg b.w.	36.40±1.51	31.00±3.67 b	30.00±1.58 b
	(35.00 – 39.00)	(27.00 - 36.00)	(28.00 - 32.00)

a,b Means within the same columns with different letters are statistically significant ($p<0.05$).

Table 4. Erythrocytes glutathione peroxidase activities in malathion treated groups (U/gHb).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	37.80±2.28 a	39.60±2.51 a	38.60±1.14 a
	(35.00 - 41.00)	(38.00 - 44.00)	(37.00 - 40.00)
100 mg/kg/day	26.40±1.14 b	23.60±1.14 b	24.20±5.06 b
	(25.00 - 28.00)	(22.00 - 25.00)	(19.00 - 30.00)
500 mg/kg/day	25.20±5.40 b	23.40±3.36 b	22.80±3.63 b
	(19.00 - 32.00)	(18.00 - 27.00)	(19.00 - 27.00)
1500 mg/kg/day	24.40±1.51 b	22.60±4103 b	21.20±3.34 b
	(22.00 - 26.00)	(18.00 - 27.00)	(17.00 – 25.00)

a,b. Means within the same columns with different letters are statistically significant ($p<0.05$)

These findings are compatible with studies of Dowla et al. (1996), Pedrajas et al. (1995) and Gromov et al. (1993). The decreases in Cu-Zn SOD and GSH-Px enzyme activities indicate that aldicarb and malathion stimulate lipid peroxidation, via the consumption of these enzymes for prevention of peroxidation cases.

Catalase activities were not significantly affected by the administration of the aldicarb (Table 5) and malathion (Table 6). Although the catalase activities slightly decreased in three aldicarb treated groups, these results were not statistically significant.

Similarly, catalase activities in malathion treated groups and malathion control group were not statistically significant different in subacute, subchronic and chronic periods. These findings were also compatible with the study of Gromov et a1.(1993), which observed that catalase activities in brain tissues were not modified by some pesticides.

Table 5. Erythrocytes catalase activities in aldicarb treated groups (k/mgHb).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	784.33±155.15	780.00±108.16	790.00±105.35
	(666.00 – 960.00)	(690.00 - 900.00)	(680.00 - 890.00)
0.3 mg/kg b.w.	621.00±303.50	833.66±167.28	787.33±369.69
	(318.00 – 925.00)	(666.00 - 1000.00)	(562.00 - 1214.00)
0.6 mg/kg b.w.	647.33±102.50	739.33±189.00	637.33±237.36
	(545.00 – 750.00)	(550.00 - 928.00)	(416.00 - 888.00)
1.2 mg/kg b.w.	625.33±40.50	713.33±119.23	664.33±420.25
	(585.00 – 666.00)	(590.00 - 828.00)	(193.00 - 1000.00)

* No significant differences as statistically between the groups in the same column ($p<0.05$).

Table 6. Erythrocytes catalase activities in malathion treated groups (k/mgHb).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	792.00±151.43	875.33±182.38	744.33±88.28
	(666.00 – 960.00)	(666.00 - 1000.00)	(585.00 - 800.00)
100 mg/kg/day	897.33±306.79	812.00±62.50	675.66±111.38
	(692.00 – 1250.00)	(750.00 - 875.00)	(585.00 - 800.00)
500 mg/kg/day	821.66±66.72	704.00±125.49	578.33±116.38
	(750.00 – 882.00)	(562.00 - 800.00)	(444.00 - 649.00)
1500 mg/kg/day	858.33±172.66	655.33±150.28	641.66±232.28
	(666.00 – 1000.00)	(500.00 - 800.00)	(375.00 - 800.00)

* No significant differences as statistically between the groups in the same column ($p<0.05$).

However, plasma MDA activities were found at high concentrations with aldicarb (Table 7) and malathion (Table 8) treatment. Malondialdehyde levels increased significantly in aldicarb treated groups after subacute, subchronic, and chronic periods, as compared to the aldicarb control group. These differences were particularly evident in group 3; the differences were not observed in the two other dose groups (group 2 and 3).

Plasma MDA levels of malathion treated groups were significantly higher than those of the malathion control group ($p<0.05$). The MDA levels increased particularly in the group 8, in subacute, subchronic and chronic periods.

These increases indicated that malathion caused to lipid peroxidation, because the MDA is the last product on this process. These differences were significant when malathion treated groups were compared to control group on subacute, subchronic and chronic periods. But in aldicarb-treated groups, the increases of MDA levels decreased with longer exposure periods, so these results show the protective effects of Cu-Zn SOD and GSH-Px enzymes for lipid peroxidation.

Table 7. Plasma malondialdehyde levels in aldicarb treated groups (nmol/ml).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	1.94±0.97 a	2.34±0.58 a	2.29±0.58 a
	(0.77 - 2.95)	(1.55 - 2.95)	(1.55 - 2.95)
0.3 mg/kg b.w.	18.81±6.62 b	21.11±9.48 b	7.72±1.03 b
	(10.26 - 25.19)	(10.11 - 33.28)	(6.82 - 9.17)
0.6 mg/kg b.w.	23.20±8.02 b	29.42±5.48 bc	8.35±1.22 b
	(14.62 - 33.28)	(21.63 - 35.77)	(7.31 – 10.11)
1.2 mg/kg b.w.	40.47±7.66 c	35.37±8.12 c	11.69±2.26 c
	(32.34 - 48.05)	(23.48 - 40.74)	(9.17 – 13.69)

a,b,c. Means within the same columns with different letters are statistically significant ($p<0.05$).

Table 8. Plasma malondialdehyde levels in malathion treated groups (nmol/ml).

Groups	Day 15 (Subacute)	Day 45 (Subchronic)	Day 120 (Chronic)
Control	1.94±0.97 a	2.37±0.58 a	1.97±0.92 a
	(0.77 - 2.95)	(1.55 - 2.95)	(0.90 - 2.95)
100 mg/kg/day	12.85±0.82 b	12.82±0.87 b	12.94±3.66 b
	(11.66 - 13.53)	(11.55 - 13.53)	(8.83 – 17.70)
500 mg/kg/day	17.10±5.71 b	15.16±1.02 b	13.56±4.02 b
	(10.26 - 24.10)	(14.15 - 16.32)	(9.64 – 17.42)
1500 mg/kg/day	23.34±1.69 c	24.14±10.01 c	24.93±13.69 c
	(21.10 - 25.19)	(13.84 - 32.81)	(15.39 - 44.84)

a,b,c. Means within the same columns with different letters are statistically significant ($p<0.05$).

High doses of aldicarb and malathion stimulated lipid peroxidation on subacute, subchronic and chronic periods. Therefore, especially MDA levels in plasma and GSH-Px and Cu-Zn SOD activities in erythrocytes were significantly changed, but there were no significant changes observed in erythrocytes catalase activities. These findings show that pesticide exposure can produce hazardous effects such as stimulation of lipid peroxidation in a mammalian test species and may be of concern to humans.

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