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Toxic Effects of Dimethoate (Organophosphate) on Metabolism and Enzyme System of Freshwater Teleost Fish *Channa punctatus*

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Abstract

Dimethoate pesticides are frequently used for pest control in agricultural fields, and may reach the surrounding freshwater bodies through irrigation or rain. These pesticides caused severe destructive effects on aquatic fauna. Fishes are the major component of aquatic fauna, which are the chief source of proteins. Carbohydrate and nitrogenous metabolism of fishes are responsible for proper growth and their development. Dimethoate pesticides are electrophilic in nature and inhibitor of acetylcholinesterase activity and they may attack other enzymes necessary for carbohydrate and nitrogenous metabolism. Freshwater fish *Channa punctatus* were treated for 24h and 96h with different nominal concentrations (i.e. 7.16 mg•L and 10.75 mg•L) of dimethoate pesticide and effect on carbohydrate and nitrogenous metabolism was observed by analyzing the different biochemical parameters in muscle, liver and gonad tissues. Carbohydrate and nitrogenous metabolism were significantly affected due to the hypoxic conditions occurred by the exposure of pesticide, as total protein, nucleic acids (DNA and RNA), glycogen, pyruvate levels and cytochrome oxidase activity were significantly decreased, while total free amino acids and lactate level and lactic dehydrogenase activity was increased after the sub-lethal exposure. So hypoxic conditions occurred by dimethoate caused severe destructive effects on carbohydrate and nitrogenous metabolism, due to which growth and development of fish was duly affected. Fishes with low protein content were not fit for human consumption.

Introduction

The next massive introduction of a new group of pesticides after organochlorine insecticides was of organophosphorus insecticides. They replaced the

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organochlorine due to their less persistent life and easy detoxification in animal tissues (Quaraishi 1977). Although other groups of insecticides with a shorter life and comparatively very low mammalian toxicity are available (e.g. pyrethroids), organophosphorus (OP) compounds are still used frequently in agricultural practices. Their extensive application may affect fish population as they enter the water through irrigation or rain (Li 1975). The OP compounds are esterase inhibitor neurotoxicants, with acute cholinergic effect preceded by inhibition of acetylcholinesterase (Barber et al. 1999). Being neurotoxicant, OP compounds interfere with many of the vital physiological functions (Rao and Rao 1983), and consequently alter the levels of various body constituents (Arasta et al. 1996; Begum and Vijayaraghavan 1996; 1999) in fishes.

Investigations have been shown that changes in carbohydrate and nitrogenous metabolism in fish induced by the stress occurred by pesticide-induced hypoxia. These changes include depletion of proteins, glycogen and pyruvate stores from fish tissues such as liver and muscle (Laul et al. 1974). Sambasiva Rao (1999) reported an elevation in free amino acids and protease activity due to pesticide-induced hypoxia. Black (1958) reported an elevation in lactic acid level in liver, muscle and blood and suggests that an uncontrolled entry of lactic acid into the tissues interferes with internal mechanisms, which maintain the acid-base balance. Lactic acid may also reduce the affinity of hemoglobin for both oxygen and carbon dioxide, diminishing the oxygen-carrying capacity of blood.

In the present study, an attempt was made to examine the sublethal toxic effect of dimethoate (OP insecticide) on carbohydrate and nitrogenous metabolism in the liver, muscle and gonad tissues of freshwater fish *Channa punctatus*. The insecticide dimethoate and fish *C. punctatus* were selected for study because the former is used often in field and the latter is an important paddy fish of Indian capture fishery.

Materials and Methods

The freshwater fish *C. punctatus* were collected from the Ramgarh lake of Gorakhpur district of Uttar Pradesh. The fish were stored in glass aquaria containing 60 L-dechlorinated tap water for seven days for acclimatization under laboratory conditions. Water was changed after every 24h. Commercial fish food was supplied to fish during acclimatization period. Dead fish (if any) were removed from the aquaria as soon as possible to avoid water fouling. Adult fish of nearly similar weight (24.0 ± 1.9 g) and length (14.5 ± 1.2 cm) were selected for experiments. Acclimatized fish were treated with 98% pure technical grade dimethoate. The IUPAC name of dimethoate pesticide is N-methylcarbamoylmethyl.

According to Srivastava and Singh (2001), LC_{50} (24h) value of dimethoate for fish *C. punctatus* is 17.92 mg•L. The fish were treated with 40% (7.16 mg•L) and 60% (10.75 mg•L) of LC_{50} (24h) of dimethoate. Six aquaria were set up for each concentration and each aquarium contained 10 fish in 10 L dechlorinated tap water. Water temperature was kept at 28 ± 1.4°C during

whole experimental period. Qualities of experimental water (pH= 7.4 ± 0.6; dissolved oxygen= 7.3 ± 0.4 ppm; free carbon dioxide= 5.8 ± 0.4 ppm; Alkalinity= 106 ± 6.8 ppm) were measured according to the method of APHA/AWWA/WPCF, (1998). Control groups were kept in dechlorinated tap water without any treatment. Fish were not fed before 24h and during the experiment. After 24h and 96h of exposure, fish were removed from aquaria and washed with freshwater. Fishes of both treated a well as control groups were killed by a severe blow on the head. Muscle, liver and gonad tissues were isolated for total protein, total free amino acids, nucleic acids (DNA and RNA), glycogen, pyruvate, lactate, lactic dehydrogenase (LDH) and cytochrome oxidase activity estimation.

Total protein

For total protein estimation, homogenates (10 mg•ml, w/v) were prepared in 10% TCA. Estimation was made according to the method of Lowry et al. (1951). Bovine serum albumin was taken as standard.

Total free amino acids

Total free amino acids were measured according to Spies (1957). Homogenate (10 mg•ml, w/v) was prepared in 96% ethanol. Glycine was taken as standard.

Nucleic acids (DNA and RNA)

DNA and RNA in gonad tissue were estimated by the method of Schneider (1957) using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg•ml, w/v) were prepared in 5% TCA at 90°C. Calf thymus DNA and yeast RNA were used as standard for DNA and RNA, respectively.

Glycogen

Glycogen was measured according to Anthrone method of Van der Vies (1954). Homogenate (10 mg•ml, w/v) was prepared in 5% TCA. Glucose was taken as standard.

Pyruvate

Pyruvate level was measured according to Friedemann and Haugen (1943). Homogenate (50 mg•ml, w/v) was prepared in 10% TCA. Sodium pyruvate was taken as standard.

Lactate

Lactate was estimated according to Barker and Summerson (1941), modified by Huckabee (1961). Homogenate (50 mg•ml, w/v) was prepared in 10% cold TCA. Sodium lactate was taken as standard.

Lactic dehydrogenase

Lactic dehydrogenase (LDH) activity was measured according to the method of Anon (1984). Homogenates (50 mg•ml, w/v) were prepared in 0.1M phosphate buffer (pH 7.5) in an ice bath. Enzyme activity has been expressed as nanomoles of pyruvate reduced/min/mg protein.

Cytochrome oxidase

Cytochrome oxidase activity was measured according to the method of Cooperstein and Lazarow (1951). Homogenates (50 mg•ml, w/v) were prepared in 0.33M phosphate buffer (pH 7.4) in an ice bath. Enzyme activity has been expressed in arbitrary units/min/mg of proteins.

Each assay was replicated six times, values are expressed as mean ± SE of six replicates, Student's 't' test was applied to locate significant ($P<0.05$ and $P<0.01$) differences between treated and control group.

Results

After the completion of 24h and 96h of exposure biochemical parameters were studied. Data of the biochemical analyzis is given in tables 1, 2, 3 and 4. Fish exposed to insecticide exhibited a significant dose-dependent decrease in total protein, nucleic acids (DNA AND RNA), glycogen, pyruvate level and cytochrome oxidase activity in liver, muscle and gonad tissues. Maximum depletion in total protein level was observed in the muscle (28% and 32% of control) and liver (28% and 32% of control) tissues of fish exposed to 24h and 96h to 10.75 mg•L of dimethoate (table 1). A significant decline in nucleic acid (DNA and RNA) level in gonad tissue of exposed fish was also observed in the present study. Maximum reduction in DNA level was found in liver tissue (61% and 66% of control), while maximally reduction in RNA level was also observed in liver tissue (72% and 76% of control) exposed to 24h and 96h to 10.75 mg•L of pesticide (table 2). Maximum decrement in glycogen level was found in liver tissues (43% and 48% of control) after exposure for 24h and 96h to 10.75 mg•L. Pyruvate level was maximally decreased in muscle tissue (71% and 76% of control) after exposure for 24h and 96h to 10.75 mg•L (table 3). Cytochrome oxidase activity maximally inhibited in liver tissue (51% and 49% of control) after exposure for 24h and 96h to 10.75 mg•L (table 4).

Total free amino acids and lactate level was significantly increased after the treatment in liver, muscle and gonad tissues. Free amino acids level was maximally increased in liver tissue (40% and 51% of control) followed by gonad and muscle tissues after exposure for 24h and 96h to 10.75 mg•L (table 1). Maximum enhancement in lactate level was found in gonad tissue (92% and 104% of control) followed by muscle and liver tissues after exposure to 24h and 96h to 10.75 mg•L of pesticide (table 3). Lactic dehydrogenase (LDH) activity was also significantly increased after the treatment in liver, muscle and gonad

Table 1. Level of total protein and total free amino acids in muscle, liver and gonad tissues of freshwater fish *C. punctatus* after exposure to nominal concentrations of 7.16 mg•L and 10.75 mg•L for 24h and 96h of dimethoate (OP).

Parameters	EP	Tissue	Control	7.16 mg•L	10.75 mg•L
Total protein (mg•mg)	24h	Muscle	156.1 ± 5.79 (100)	134.6 ± 4.26^{+} (86)	112.8 ± 2.83^{+} (72)
		Liver	138.2 ± 3.41 (100)	112.8 ± 3.45^{+} (82)	99.9 ± 2.84^{+} (72)
		Gonad	146.5 ± 4.76 (100)	131.4 ± 2.47^{+} (90)	109.9 ± 3.69^{+} (75)
	96h	Muscle	156.8 ± 5.74 (100)	127.8 ± 4.36^{+} (81)	106.2 ± 2.63^{+} (68)
		Liver	138.2 ± 3.41 (100)	106.1 ± 3.16^{+} (77)	94.7 ± 2.70^{+} (68)
		Gonad	146.5 ± 4.76 (100)	124.3 ± 2.89^{+} (85)	103.7 ± 3.12^{+} (71)
Total free amino acids (mg•mg)	24h	Muscle	26.86 ± 1.04 (100)	28.35 ± 1.08 (106)	32.85 ± 1.08^{+} (122)
		Liver	21.48 ± 1.24 (100)	24.37 ± 1.04^{+} (113)	30.12 ± 1.07^{+} (140)
		Gonad	23.34 ± 1.08 (100)	25.48 ± 1.15^{+} (109)	30.74 ± 1.12^{+} (132)
	96h	Muscle	26.86 ± 1.04 (100)	29.49 ± 1.06^{+} (110)	36.32 ± 1.09^{+} (135)
		Liver	21.48 ± 1.24 (100)	25.52 ± 1.11^{+} (119)	32.43 ± 1.09^{+} (151)
		Gonad	23.34 ± 1.08 (100)	26.18 ± 1.19^{+} (112)	33.46 ± 1.24^{+} (144)

EP = Exposure period (in hours)
Values are mean ± SE of six replicates
$^{+}$ = ($P<0.05$); $^{++}$ = ($P<0.01$), when Student's 't' test was applied between control and treated groups
Values given in parenthesis are percent change with control taken as 100%

tissues. Maximum increment in activity of lactic dehydrogenase was observed in gonad tissue (108% and 142% of control) followed by liver and muscle tissue after exposure for 24h and 96h to 10.75 mg•L of dimethoate (table 4).

Discussion

Proteins are mainly involved in the architecture of the cell. During chronic period of stress they are also a source of energy (Umminger 1977). Behavioural responses of fish exposed to sublethal concentration of dimethoate showed that they were under stress condition. During stress condition, fish needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative source of energy is protein to meet the increased energy demand. The depletion of protein fraction in liver, muscle and gonad tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Other workers such as Malla Reddy and Bashamohideen (1995); Singh et al. (1996) have also reported decline in protein constituent in different fish tissue

Table 2. Level of nucleic acids (DNA and RNA) in muscle, liver and gonad tissues of freshwater fish *C. punctatus* after exposure to nominal concentrations of 7.16 mg•L and 10.75 mg•L for 24h and 96h of dimethoate (OP).

Parameters	EP	Tissue	Control	7.16 mg•L	10.75 mg•L
DNA (µg•mg)	24h	Muscle	109.8 ± 3.08 (100)	66.72 ± 2.43^{+} (61)	46.25 ± 2.14^{+} (42)
		Liver	122.7 ± 3.18 (100)	74.62 ± 2.69^{+} (61)	47.75 ± 2.09^{++} (39)
		Gonad	147.9 ± 5.29 (100)	73.76 ± 3.86^{+} (50)	59.29 ± 3.69^{+} (40)
	96h	Muscle	109.2 ± 3.05 (100)	63.49 ± 2.38^{+} (58)	41.78 ± 2.02^{++} (38)
		Liver	123.6 ± 3.14 (100)	69.69 ± 2.67^{+} (56)	42.24 ± 2.04^{++} (34)
		Gonad	147.0 ± 5.04 (100)	69.50 ± 3.25^{+} (47)	53.83 ± 3.42^{++} (37)
RNA (µg•mg)	24h	Muscle	87.68 ± 2.57 (100)	38.72 ± 2.45^{++} (44)	26.86 ± 2.77^{++} (31)
		Liver	98.75 ± 3.27 (100)	38.65 ± 2.36^{++} (39)	27.93 ± 2.12^{++} (28)
		Gonad	108.13 ± 3.74 (100)	41.23 ± 2.46^{++} (38)	32.32 ± 2.11^{++} (30)
	96h	Muscle	87.23 ± 2.45 (100)	34.12 ± 2.12^{++} (39)	22.34 ± 2.70^{++} (26)
		Liver	98.45 ± 3.21 (100)	35.34 ± 2.13^{++} (36)	24.12 ± 2.46^{++} (24)
		Gonad	107.6 ± 3.25 (100)	37.00 ± 2.40^{++} (34)	28.32 ± 2.23^{++} (26)

Details are as given in table 1.

exposed to sublethal concentrations of insecticides. Furthermore, any obstruction in RNA synthesis may also affect protein level as it plays an important role in protein synthesis. In the present investigation, a significant decline in RNA level was also observed in treated fish.

Increment in free amino acids level was the result of breakdown of protein for energy requirement and impaired incorporation of amino acids in protein synthesis (Singh et al. 1996). It also attributed to lesser use of amino acids (Seshagiri Rao et al. 1987) and their involvement in the maintenance of an acid-base balance (Moorthy et al. 1984). Stress conditions induce elevation in the transamination pathway (Natrajan 1985). Inhibition of RNA synthesis may also affect protein and amino acid levels. Inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. Dimethoate pesticide appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the OP compounds may attack many enzymes responsible for normal metabolic pathway. Thus, it is possible that the enzyme necessary for DNA synthesis might have been inhibited by this insecticide. On compilation of the results, it appears that the disruption of DNA synthesis might have affected RNA synthesis and consequently protein synthesis.

Table 3. Level of glycogen, lactate and pyruvate in muscle, liver and gonad tissues of freshwater fish *C. punctatus* after exposure to nominal concentrations of 7.16 mg•L and 10.75 mg•L for 24h and 96h of dimethoate (OP).

Parameters	EP	Tissue	Control	7.16 mg•L	10.75 mg•L
Glycogen (mg•g)	24h	Muscle	1.84 ± 0.41 (100)	1.42 ± 0.23^{+} (77)	1.17 ± 0.19^{+} (64)
		Liver	1.99 ± 0.47 (100)	1.43 ± 0.34^{+} (72)	1.14 ± 0.21^{+} (57)
		Gonad	1.72 ± 0.54 (100)	1.34 ± 0.37^{+} (78)	1.21 ± 0.19^{+} (70)
	96h	Muscle	1.72 ± 0.12 (100)	1.21 ± 0.10^{+} (70)	1.01 ± 0.11^{+} (58)
		Liver	1.96 ± 0.15 (100)	1.29 ± 0.14^{+} (66)	1.02 ± 0.13^{+} (52)
		Gonad	1.68 ± 0.18 (100)	1.24 ± 0.17^{+} (74)	0.98 ± 0.11^{+} (58)
Lactate (mg•g)	24h	Muscle	2.54 ± 0.32 (100)	3.21 ± 0.54^{+} (126)	4.63 ± 0.58^{+} (182)
		Liver	2.49 ± 0.34 (100)	3.15 ± 0.62^{+} (126)	4.41 ± 0.58^{+} (177)
		Gonad	2.24 ± 0.42 (100)	3.02 ± 0.51^{+} (135)	4.30 ± 0.59^{++} (192)
	96h	Muscle	2.56 ± 0.39 (100)	3.55 ± 0.47^{+} (139)	4.84 ± 0.29^{+} (189)
		Liver	2.50 ± 0.28 (100)	3.21 ± 0.34^{+} (128)	4.61 ± 0.54^{+} (184)
		Gonad	2.26 ± 0.42 (100)	3.14 ± 0.46^{+} (139)	4.62 ± 0.67^{++} (204)
Pyruvate (µ moles•g)	24h	Muscle	1.56 ± 0.25 (100)	0.70 ± 0.14^{+} (45)	0.46 ± 0.15^{++} (29)
		Liver	1.37 ± 0.44 (100)	0.69 ± 0.15^{+} (50)	0.42 ± 0.15^{++} (31)
		Gonad	1.29 ± 0.22 (100)	0.85 ± 0.16^{+} (66)	0.53 ± 0.15^{+} (41)
	96h	Muscle	1.54 ± 0.52 (100)	0.62 ± 0.16^{+} (40)	0.37 ± 0.18^{++} (24)
		Liver	1.36 ± 0.48 (100)	0.64 ± 0.15^{+} (47)	0.38 ± 0.17^{++} (28)
		Gonad	1.28 ± 0.44 (100)	0.73 ± 0.17^{+} (57)	0.44 ± 0.18^{++} (34)

Details are as given in table 1.

Carbohydrates are the primary and immediate sources of energy. Arasta et al. (1996) suggested in stress condition, carbohydrates reserve depleted to meet energy demand. Depletion of glycogen may be due to direct utilization for energy generation, a demand caused by pyrethroid-induced hypoxia. Since dimethoate is the inhibitor of acetylcholinesterase. Inhibition of acetylcholinesterase results in as increase in acetylcholine contents (Singh et al. 1996). Increased level of latter has been shown to enhance the secretion of catecholamine in fish (Nilsson et al. 1976), which may bring about glycogenolysis. Finally, liver, muscle and gonad glycogenolysis seem to be the result of increased secretion of catecholamine due to stress of insecticide treatment (Singh and Srivastava 1992; Singh and Agarwal 1993). Decrement in pyruvate level is due to higher energy demand during pesticidal exposure. In consonance with the

Table 4. Activities of Lactic dehydrogenase (LDH) and Cytochrome oxidase in muscle, liver and gonad tissues of freshwater fish *C. punctatus* after exposure to nominal concentrations of 7.16 mg•L and 10.75 mg•L for 24h and 96h of dimethoate (OP).

Parameters	EP	Tissue	Control	7.16 mg•L	10.75 mg•L
LDH (μ moles•mg protein•h)	24h	Muscle	0.10 ± 0.04 (100)	0.15 ± 0.09^{+} (146)	0.21 ± 0.12^{++} (205)
		Liver	0.10 ± 0.016 (100)	0.13 ± 0.09^{+} (129)	0.18 ± 0.14^{++} (182)
		Gonad	0.08 ± 0.004 (100)	0.13 ± 0.014^{+} (153)	0.17 ± 0.12^{++} (208)
	96h	Muscle	0.10 ± 0.02 (100)	0.18 ± 0.08^{+} (169)	0.25 ± 0.10^{++} (237)
		Liver	0.10 ± 0.015 (100)	0.16 ± 0.07^{+} (158)	0.22 ± 0.13^{++} (219)
		Gonad	0.09 ± 0.003 (100)	0.14 ± 0.017^{+} (161)	0.22 ± 0.11^{++} (242)
Cytochrome oxidase (arbitrary units• min•mg protein)	24h	Muscle	80.10 ± 9.04 (100)	67.34 ± 5.68^{+} (84)	54.87 ± 6.54^{+} (68)
		Liver	83.99 ± 10.16 (100)	56.45 ± 3.47^{+} (67)	42.79 ± 9.38^{+} (51)
		Gonad	78.37 ± 8.09 (100)	65.79 ± 7.78^{+} (84)	48.25 ± 8.88^{+} (62)
	96h	Muscle	79.04 ± 8.72 (100)	64.22 ± 7.48^{+} (81)	49.23 ± 7.566^{+} (62)
		Liver	82.49 ± 9.15 (100)	52.23 ± 8.33^{+} (63)	40.34 ± 6.33^{+} (49)
		Gonad	76.82 ± 7.03 (100)	62.19 ± 8.13^{+} (81)	46.29 ± 8.26^{+} (60)

Details are as given in table 1.

increase in lactate content there is a decrease in the pyruvate level and this trend has been observed in both the tissues. The decrease in pyruvate level suggests the possibility of a shift towards anaerobic dependence due to a remarkable drop in aerobic segment. The decrease in pyruvate could be due to its conversion to lactate, or due to its mobilization to form amino acids, lipids, triglycerides and glycogen synthesis in addition to its role as a detoxification factor in ammonia toxicity (Sambasiva Rao 1999). The increase in lactate corroborates with the corresponding decrease in pyruvate content of the tissues of fish exposed to dimethoate. The increase in lactate also suggests a shift towards anaerobiosis as a consequence of hypoxia created under pesticide toxic impact leading to respiratory distress (Domsche et al. 1971; Sambasiva Rao 1999). Liver, muscle and gonad tissues receive less oxygen, leading to severe tissue hypoxia. Huckabee (1958) reported that an upward trend in lactate in the tissues might be taken to indicate that oxygen supply to the tissues is not adequate for the normal metabolic function. Development of such internal hypoxic conditions may be ultimately responsible for the shift to the less efficient anaerobic metabolism, evidenced by the change in lactate content observed during this study.

Lactic dehydrogenase (LDH) forms the center for a delicately balanced equilibrium between catabolism and anabolism of carbohydrates (Everse and Kaplan 1973). Stimulation of LDH and the rapid rate of glycolysis observed in

the present study indicate that the end product of glycolysis i.e. pyruvate, was not routed through Kreb's cycle but through the lactic acid cycle under hypoxic conditions, leading to the accumulation of lactic acid. Ghosh (1987) reported similar observations in liver and muscle of *Clarias batrachus* exposed to three OP pesticides, adding substantial support to the present observations. Inhibition in cytochrome oxidase supports that dimethoate pesticide shown a profound impact on the oxidative metabolism, possibly due to their influence on respiratory process like electron transport system (ETS). Decrease in cytochrome oxidase might be either the result of reduced availability of O_2, which in turn has reduced the capacity of ETS to produce ATP molecules or should be due to the direct impact of the pesticide. Conney (1967) and Stevans et al. (1972) reported that anticholinesterase compounds are known to usually inhibit mitochondrial reactions like the function of the cytochrome oxidase in ETS. So dimethoate poisoning effecting Kreb's cycle thereby diminishes the rate of ETS and oxidative phosphorylation, resulting in less synthesis of ATP.

Conclusion

In conclusion, it can be stated that dimethoate poisoning may lead to alteration in carbohydrate and nitrogenous metabolism in fish. Fish with low protein values (due to insecticide pollution) is not fit for human consumption. This implies that one should take the necessary precaution in the application of insecticides to protect the life of fish and other aquatic fauna.

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