ejpmr, 2016,3(11), 429-434

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ASSESSMENT OF ACUTE TOXICITY AND BEHAVIORAL CHANGES IN FRESHWATER FISH *CATLA CATLA* EXPOSED TO ORGANOPHOSPHATE PESTICIDE PHORATE 10% CG

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Article Received on 05/09/2016 **Article Revised on 25/09/2016** **Article Accepted on 15/10/2016**

ABSTRACT

The present study is to assess the acute toxicity and behavioral alterations of Phorate 10% CG {O, O-diethyl-S-[(ethylthio) methyl] phosphorodithioate}, an organophosphate pesticide on *Catla catla*. The most common acute toxicity test is acute lethality and LC_{50} is customary to represent the lethality of a test species in terms of mortality and time. The healthy juveniles of fish *C. catla* were used for this study. The water quality parameters were in normal range, indicating good water quality for experimentation. The Median lethal concentration values of *C. catla* for Phorate in static tests for 24, 48, 72 and 96h were 0.81, 0.76, 0.68 and 0.53 mg/L respectively. Zero percent mortality of the fish was observed at the concentration of the toxicant at and below0.54, 0.48 0.24 and 0.30 mg/l for 1, 2, 3 and 4 days. 100 percent mortality of the fish was observed at the concentration of the toxicant at and above 1.60, 1.10, 0.96 and 0.79 mg/l for 1, 2, 3 and 4 days respectively. In the present study, irregular swimming, loss of balance, restlessness, excess secretion of mucous and surfacing activity with gradual decrease in opercular movement was the common observations. There were no deaths and behavioral changes observed in the control group throughout the experiment.

KEYWORDS: Phorate, *Catla catla*, LC_{50}, Mortality, Behavioral changes.

INTRODUCTION

Environmental pollution resulting from industrial effluents and agricultural activities has become a global issue because of the extent damage caused to the aquatic ecosystems and the disruption in the natural food chain, by several agricultural practices such as insecticidal and herbicidal application. The increasing population is generating a stress on resources, resulting in the excessive use of organophosphorus pesticides and fertilizers to meet the demand. These substances ultimately pollute the aquatic environment and cause severe damage to the aquatic life especially the non - target species. Among the different groups of pesticides organophosphates are being used commonly as insecticides due to their facilitation properties like low mammalian toxicity, less persistence and rapid biodegradability in nature.[1] Phorate {O,O-diethyl-S-[(ethylthio) methyl] phosphorodithioate} is an organophosphate pesticide used in agricultural practices, primarily to control sap-feeding insects which includes various beetles, mites, grubs and worms. This compound has been found to produce a lot of toxic effects in different fish species and is able to bring about changes in their metabolic pathways. Phorate is widely used throughout the world and also in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops. Commercial names of phorate are Thimet, Rampart, Granutox Agrimet etc. and its molecular formula is $C_7 H_{17} O_2 PS_3$.[2]

Acute toxicity of a pesticide refers to the chemical's ability to cause injury to an animal from a single exposure, generally of short duration. The acute toxicity test of pesticides to fish has been widely used to acquire rapid estimates of the concentrations that cause direct, irreversible harm to test organisms.[3] The most common acute toxicity test is acute lethality and LC_{50} is customary to represent the lethality of a test species in terms of mortality and time. LC_{50} is the concentration of the chemical that results in the 50% death rate of the test organisms.

Water is one of the most essential needs for the survival of life on earth. Water covers 71% of the earth's surface and is vital for all forms of life. The aquatic environment is currently under threat by the indiscriminate use of

synthetic pesticides by the human activities and causing high risk to non-target organisms including fish. Pesticides used for controlling pests in agriculture are one of the major causes of aquatic pollution. Heavy dependence of modern agriculture on agrochemicals such as pesticides is emerging as a threat to the ecological balance of aquatic ecosystems. Pesticides are carried into the aquatic ecosystems by surface runoff from sites of application and therefore the health of aquatic ecosystem is being adversely affected because they serve as an ultimate sink for these pesticides. These pesticides are also found to be highly toxic not only to fish but also to other organisms which constitute food of the fish. Among synthetic pesticides, organophosphates are widely used in agriculture and in health and hygiene.[2]

MATERIALS AND METHODS

Experimental Fish

The healthy juveniles of fish *C. catla* with body length ranging 7–9 cm and weighing 6 – 8 gm (N=50) were obtained from local fish farm at Buddam in Guntur district in Andhra Pradesh state, India. Prior to conducting experiment, the fish were acclimatized for a period of 10 to 15 days in dechlorinated tap water under laboratory conditions at room temperature (28 ± 2^{o}C). The container was aerated with rich oxygen. Hygienic conditions were maintained by renewed water regularly and fish were daily fed with supplementary feed consisting of rice bran and fish pellets.

Water Quality Analysis

The physico-chemical parameters of water such as temperature, turbidity, pH, total hardness, total suspended solids, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), sulphates and Dissolved Oxygen were estimated during the course of the study according to the standard protocols APHA.[4]

Pesticide

The commercial grade formulations of Phorate 10% CG (Encapsulated 10% Granules) an organophosphate pesticide is used as a toxicant in the present experiment. Commercial names of phorate are Tiger 10, Thimet, Rampart, Granutox Agrimet etc. and its molecular formula is $C_7H_{17}O_2PS_3$, purchased from local pesticide market of Guntur in Andhra Pradesh, India.

Preparation of Stock Solution

Stock solution of phorate was prepared by dissolving 1 gram of pesticide in 100 ml of acetone and the required quantity of phorate was drawn from the stock solution to maintain the suitable concentration of 1 mgl^{-1} in the container. The fish were separated into several groups and each containing 10 individuals. Pilot experiments were conducted to derive the LC_{50} determinations. These groups were exposed to different concentrations for acute toxicity estimation range from 0.24 mg/L to 1.60 mg/L for 24, 48, 72 and 96hrs. During the whole experiment a control group was maintained with acetone for comparison. The experimental design included three replicates. The fish were not fed on the day before the beginning of the experiment.

The concentration of the pesticide that caused 50% mortality at 96h was taken as the LC_{50} value in the test organisms. Each day number of dead fish were counted and removed immediately from the test container. Percent mortality was carried out and the values were pooled up into probit scale. These values were determined and analyzed by using Finney's Probit analysis method.[5]

Statistical Analysis: The data statistically were analyzed by Minitab 16.0v statistical package.

RESULTS AND DISCUSSION

Before the start of experiment, the water used for maintenance of fish was analyzed for its quality. Thus, Table 1 gives the values of different water quality parameters which are in normal range indicating good water quality for experimentation.

Table 1: Estimation of physico-chemical parameters of water used for experimentation

Water Parameter	Value
Temperature	28 ± 2^{0}C
Turbidity	7.3 silica units
P^{H} value at 28^{0}c	7.20
Total Hardness as($CaCo_3$)	160 (mg/L)
Total suspended solids (TSS)	3 (mg/L)
Conductivity	178(mg/L)
BOD	7-11 ppm
Sulphates (SO_4)	Trace amounts
Phosphates (PO_4)	Trace amount
Dissolved Oxygen(DO)	6-7 mg/L

In the present study, the fishes (C. *catla)* were subjected to various concentration of Phorate and their behavior pattern was observed after exposure to the toxicant. Initially, the fishes were hitting against the container walls and started disturbing themselves. Irregular swimming, loss of balance, restlessness, excess secretion of mucous and surfacing activity with gradual decrease in opercular movement was the common observations in the present study. With the increased exposure, the gills changed from reddish to brownish colour and the body of the fish observed to be pale in colour. Moreover the secretion of mucous has been increased before mortality of fish which shows that *C.catla* has undergone pesticidal stress. During the experiment the treated fish body weight gradually decreased when compared with the control fish. Therefore, the present study could be taken as an indicator of aquatic pollution.

The percentage of mortality rate was observed at 24, 48, 72 and 96h at different concentrations of the toxicant. Zero percent mortality of the fish was observed at the concentration of the toxicant at and below 0.54, 0.48 0.24 and 0.30 mg/l for 1, 2, 3 and 4 days respectively. Hundred percent mortality of the fish was observed at the concentration of the toxicant at and above 1.60, 1.10, 0.96 and 0.79 mg/l for 1, 2, 3 and 4 days (Table 2 -5) respectively. There were no deaths and behavioral changes observed in the control group throughout the experiment. The theoretical spontaneous response in the control group was zero. The Median lethal concentration values of *C. catla* for Phorate in static tests for 24, 48, 72 and 96h were shown in (Table 2 - 5). Thus the average LC_{50} for 96h is determined to be 0.53 mg/l, 95% upper and lower confidence limits were found to be 0.41-0.63 mg/l, respectively. The mortality rate was gradually increased, as the concentration of the toxicant increased in the experiment. Table 6, shows the estimated values of the LC_{50} for 24, 48, 72 and 96h as 0.81, 0.76, 0.68 and 0.53 mg/L respectively.

Table 2: Effect of Phorate on survival of *Catla catla* for 24hours

S. No.	Conc. of toxicant(mg/L)	Log Conc.	No. of Exposed	No. of Dead	Per. of mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.54	-0.2676	10	0	0	--
2	0.60	-0.2218	10	0	0	--
3	0.65	-0.1870	10	1	10	3.72
4	0.70	-0.1549	10	3	30	4.48
5	**0.81**	-0.0915	10	5	50	5.00
6	0.88	-0.0555	10	6	60	5.25
7	0.96	-0.0177	10	7	70	5.52
8	1.20	0.07918	10	8	80	5.84
9	1.38	0.1398	10	9	90	6.28
10	1.60	0.2041	10	10	100	8.09

Table 3: Effect of Phorate on survival of *Catla catla* for 48hours

S. No.	Conc. of toxicant(mg/L)	Log Conc.	No. of Exposed	No. of Dead	Per. of mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.48	-0.3187	10	0	0	---
2	0.56	-0.2518	10	1	10	3.72
3	0.61	-0.2146	10	3	30	4.48
4	0.68	-0.1674	10	4	40	4.75
5	**0.76**	-0.1191	10	5	50	5.00
6	0.81	-0.0915	10	6	60	5.25
7	0.87	-0.0604	10	7	70	5.52
8	0.94	-0.0268	10	8	80	5.84
9	0.99	-0.0043	10	9	90	6.28
10	1.10	0.04139	10	10	100	8.09

Table 4: Effect of Phorate on survival of *Catla catla* for 72 hours

S. No.	Conc. of toxicant (mg/L)	Log Conc.	No. of Exposed	No. of Dead	Per. of mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.24	-0.6197	10	0	0	---
2	0.31	-0.5086	10	1	10	3.72
3	0.39	-0.4089	10	2	20	4.16
4	0.52	-0.2839	10	4	40	4.75

5	**0.68**	-0.1674	10	5	50	5.00
6	0.72	-0.1426	10	6	60	5.25
7	0.79	-0.1023	10	7	70	5.52
8	0.83	-0.0809	10	8	80	5.84
9	0.90	-0.0457	10	9	90	6.28
10	0.96	-0.0177	10	10	100	8.09

Table 5: Effect of Phorate on survival of *Catla catla* for 96 hours

S. No.	Conc. of toxicant (mg/L)	Log Conc.	No. of Exposed	No. of Dead	Per. of mortality	Probit Mortality
Control	-------		10	0	0	---
1	0.30	-0.5228	10	0	0	---
2	0.36	-0.4436	10	2	20	4.16
3	0.40	-0.3979	10	3	30	4.48
4	0.48	-0.3187	10	4	40	4.75
5	**0.53**	-0.2757	10	5	50	5.00
6	0.59	-0.2291	10	6	60	5.25
7	0.64	-0.1938	10	7	70	5.52
8	0.70	-0.1549	10	8	80	5.84
9	0.75	-0.1249	10	9	90	6.28
10	0.79	-0.1023	10	10	100	8.09

Table 6: Estimated LC_{50} values and confidence limits of fish *Catla catla*

Time of Exposure	Toxicant Conc. (mg/l)**	95% Confidence Intervals(CI)
		UCL* - LCL*
24h	0.81	0.61-1.01
48h	0.76	0.52-0.99
72h	0.68	0.44-0.91
96h	0.53	0.41-0.63

** 24-96hrs LC_{50} values, *UCL= Upper confidence limits, *LCL= Lower confidence limits

Earlier studies revealed that the LC_{50} of a chemical for a species may vary under different environmental condition like time of exposure, size and other impacts. Several reports were given for different LC_{50} values of various pesticides on fresh water fish.[6-10]

The other impact of the pesticide could be observed by the behavioral changes like surfacing, erratic movement, increased mucous secretion, decreased opercular movement and loss of balance. Similar observations were made by Shivakumar *et al*.,[11] in *L. rohita* when exposed to endosulfan. Atchison and Santherinrich[12] has observed erratic and exited movements with increasing fin flickers. The erratic swimming of the treated fish indicates that the loss of physiological equilibrium and the hyper-excitability of the fish invariably in the lethal and sublethal exposure of chemical may be due to the inhibition of cholinesterase.[13] Abnormal swimming and loss of balance was caused by the deficiency in nervous and muscular coordination.

Opercular movement has been decreased with increase in toxicant concentration and accumulation of more fecal matter was observed in the container and similar results were observed by Imtiyaz Ahmad Bhatt *et al*.[14] The fast opercular movements might be due to accumulation of mucus over gills to the pesticide and similar observation was expressed by Prasanth *et al*.[15] Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Behavioral changes of *L. rohita* have been studied for various chemicals by Marigoudar *et al*.[16] and Pandey *et al*.[17] The treated fishes also showed fading of their body color before death, these changes can be considered as symptoms of stress on account of the toxicological nature of the environment. The behavioral changes showed by the fishes after Phenthoate intoxication are similar to those observed in other fishes exposed to organophosphate pesticides.[18] Bayne *et al*.[19] reported that altered movement of *Channa gachua* at different concentrations of dimethoate. The surfacing phenomenon of fish might be due to hydro toxic condition of the fish; these results are supported by Appa Rao *et al*.[20] and Charjan *et al*.[21] The decrease in body weight could be due to excessive expenditure of more energy on metabolism in fish growth and it was proportionate to the concentration of the pesticides. Similar results were reported by Balasubramani *et al*.[22] and Cook *et al*.[23]

Phorate has been reported to be highly toxic to non targeted organisms. In India, the effects of phorate were studied on circulating leucocytes of *Channa punctatus*. The hemoglobin levels, as well as the number of small lymphocytes decreased while the number of large lymphocytes, thrombocytes and total leucocytes counts increased in the fish. In an overall response against the

pesticide, the fish showed restlessness, rapid body movement, convulsions, difficulty in respiration, excess mucous secretion, change in colour and loss of balance on exposure to pesticides. This pesticide has also been reported to cause disorders in carbohydrate and lipid metabolism in *Clarias batrachus*, a freshwater fish. It depleted the cholesterol level and elevated the amount of alkaline phosphatase and bilirubin in the blood serum L.[24]

In the present investigation, Phorate caused 100% mortality of *C. catla* at 0.79, mg/l, 50% mortality at 0.53 mg/l during 96h exposure and insecticidal toxicity influenced by factors like temperature, size etc. Kumaravel[25] has reported monocrotopas caused 100% mortality of *L. rohita* at 0.0044 and 50% at 0.0036 ppm and also suggested that the lambda cyhalothrin caused mortality 100% at 0.0029 and 50% at 0.0021 mg/l. Intiyaz Ahhmad Bhat *et al* [14] determined the 96h LC_{50} value of Kethrin to *L. rohita* as 21.68 ppm. Shukla reported the LC_{50} value of dimethoate for *Colisa fasciatus* as 13.0 mg/l for 24h, 11.4 mg/l for 48h, 10.0 mg/l for 72h and 9.3 mg/l for 96h.[26]

Mohanty et al. evaluated tissue specific genotoxic effects of phorate in Labeo rohita and appraise the in vivo DNA repair ability of the fish. *Labeo rohita* (rohu) fingerlings were exposed to different concentrations (0.001, 0.002 and 0.01 ppm) of phorate, an organophosphate pesticide; samplings were done at 24, 48, 72 and 96 h. The study was carried out to evaluate tissue specific genotoxic effects produced by phorate, on three different tissue systems and to assess DNA repair response in fish. Results of tissue specific DNA damage experiments showed low baseline damage in blood cells followed by gill and liver cells in control individuals whereas more DNA breaks were found in liver followed by gill and blood cells of treated individuals.[24]

Ghazala et al reported the acute toxicity of organophosphates and carbamates on *Catla catla* fingerlings. In his study he has reported the acute effects of commercial formulation of triazophos, profenofos, carbofuran and carbaryl. Pesticides were applied to fingerlings that had been grown under optimized standard conditions under a maintained static bioassay system. Probit analysis was used for the estimation of LC50 values, which were ascertained as 4.84, 0.19, 0.99 and 7.89 mg/L for triazophos, profenofos, carbofuran and carbaryl, respectively. 100% mortality of *Catla catla* was observed with a 2.8 mg/L dose of carbofuran at 96 hours with a significant difference. Acute toxic stress was noticed with subjects exhibiting behavioral intoxication, including suffocation, lying on the bottom, erratic swimming, lethargy and downward movements and gulping prior to mortality.[27]

Sana k et al. reported acute toxicity of Phorate on fish Labeo rohita along with behavioral and biochemical changes. In his studies he reported that at a concentration of 0.137 mg/L for 96 hours there was acute toxicity. During his study, he observed rohu fingerlings were very sensitive to Phorate. He also observed behavioral responses such as erratic swimming, falls in opercular activity and increased gulping of air to meet the respiratory requirements.[28]

Hussain et al. investigated the acute toxicity of Dimethoate, an Organophosphate insecticide and its response on behavior of *Catla catla* using biochemical parameters of blood which were carried out in a static renewal system. The LC50 values of Dimethoate at various exposure times were 21.0mg/l for about 84 h; 21.5mg/l for 72 h; 22.0mg/l for about 60h; 22.5mg/l for 48 h; 23.0mg/l for 48 h; and 23.5mg/l for 24 h. The *Catla catla* showed behavioral alterations against Dimethoate intoxication viz, uncoordinated movements, erratic swimming, convulsions, excess mucus secretion, decreased opercular movements, loss of balance, drowning and change in body pigmentation, muscle fasciculation, moribund lethargy, refusal of feeding and respiratory distress. These symptoms became more apparent with increase in duration of exposure at all test concentration of Dimethoate. These results closely related to our present reports.[29]

From this study it could be concluded that the excess use of chemical pesticides not only results in the extermination of the target organisms but also of a large number of non-target organisms affected in such a way that their normal physiological mechanisms are hampered. This results in the death of the fishes there by decreasing their population and bringing imbalance in ecosystem. Thus this study could be used as a tool for creating awareness among the local farmers so that the use of these deadly pesticides could be minimized.

CONCLUSION

In conclusions our study shows that phorate is most toxic to the experimental fish and it has negatively acted on fish behaviour. This work finally concludes that the usage of Phorate is not safe for ecosystem and non target organisms.

ACKNOWLEDGEMENT

The authors are thankful to the Coordinator, Department of Biochemistry, Zoology and Aquaculture, Acharya Nagarjuna University for providing laboratory facilities.

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