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Morpho-toxicology of chlorpyrifos to prolactin cells of a freshwater catfish, *Heteropneustes fossilis*

Ajai Kumar Srivastav*, Sanjay Kumar Srivastava, Sarojni Tripathi, Diwakar Mishra and Sunil Kumar Srivastav

*Department of the Zoology, University Gorakhpur, 273 009, Gorakhpur, India. *Author for correspondence. E-mail: ajaiksrivastav@hotmail.com*

ABSTRACT. In the present study, an organophosphorus compound Coroban (active ingredient chlorpyrifos – E.C. 20%) was used. In short-term exposure the fish were subjected to 0.8 of 96h LC_{50} value of chlorpyrifos (1.76 mg L^{-1}) for 96h. In long-term exposure the experiment was performed for 28 days by using 0.2 of 96h LC_{50} value of chlorpyrifos (0.44 mg L^{-1}). Fish were killed on each time intervals from control and experimental (chlorpyrifos) groups after 24, 48, 72, and 96h in short-term exposure and after 7, 14, 21, and 28 days in long-term experiment. Blood samples were collected and sera were analyzed for calcium. Pituitary glands were fixed for histological studies and stained with Herlant tetrachrome and Heidenhain's azan techniques. Short-term exposure of chlorpyrifos caused decrease in the serum calcium levels. No change was noticed in the prolactin cells of chlorpyrifos treated fish. Long-term treatment with chlorpyrifos provoked hypocalcemia. The prolactin cells of treated fish exhibited slight degranulation after 21 days whereas the nuclear volume remained unchanged. After 28 days, the prolactin cells exhibited further degranulation and the nuclear volume recorded an increase. Cytolysis and vacuolization were also visible.

Keywords: prolactin cells, serum calcium, organophosphate, chlorpyrifos, fish.

Morfotoxicologia de clorpirifos para as células de prolactina de bagre de água doce *Heteropneustes fossilis*

RESUMO. No estudo presente, o composto organofosforo Coroban (ingrediente ativo clorpirifo – E.C. 20%) foi usado. Na exposição a curto prazo os peixes foram submetido a 0,8 de valor LC_{50} de 96h de clorpirifo (1,76 mg L^{-1}) durante 96h. Na exposição a longo prazo o experimento foi executado durante 28 dias usando 0,2 de valor LC_{50} de 96h de clorpirifos (0,44 mg L^{-1}). Os peixes foram mortos a cada intervalo dos grupos controle e experimental (clorpirifos) após 24, 48, 72, e 96h em exposição a curto prazo e após 7, 14, 21, e 28 dias no experimento a longo prazo. As amostras de sangue foram colhidas e o soro foi analisado para cálcio. As glândulas pituitárias foram fixadas para estudos histológicos e colorido por tetracromo de Herlant e por técnicas de azan do Heidenhain. A exposição a curto prazo do clorpirifo diminuiu os níveis de cálcio no soro. Nenhuma mudança foi observada nas células de prolactina nos peixes tratados com clorpirifo. O tratamento a longo prazo com clorpirifo causou hipocalcemia. As células de prolactina dos peixes tratados mostraram uma leve degranulação após 21 dias ao passo que o volume nuclear permaneceu inalterado. Depois de 28 dias, as células de prolactina mostraram mais degranulação e o volume nuclear registrou um aumento. Citólise e vacuolização também eram visíveis.

Palavras-chave: celulas de prolactina, calico serico, organofosfato, chlorpyrifos, peixes.

Introduction

Pesticides are being used by the human beings for their benefits - control of insect vectors of diseases and increased yield of many crops. The use of pesticides has caused severe environmental and health hazards to organisms including human beings (ABDOLLAHI et al., 2004; TUZMEN et al., 2008). Persistence of organochlorine pesticides in the environment resulted in the search for less persistent pesticide - the organophosphorus (OP) compounds. The first OP compounds developed were tepp and parathion, followed by malathion. Now there are scores of OP compounds, some liquids and some solids, mainly in the molecular forms known as phosphorothioates, phosphoro-dithioates and phosphates.

Chlorpyrifos [O, O-diethyl-O- (3, 5, 6-trichloro-2-pyridil) phosphoro-thioate], is a member of organophosphate class of insecticides that displays broad-spectrum insecticidal activity against a number of important arthropod pests (RACKE, 1993). It is recommended as a most effective insecticide for the control of mustard aphid, *Liapaphis erysimi* (SRIVASTAVA; SRIVASTAVA,

1986). The wide-spread use of chlorpyrifos is probably due to its relatively low mammalian toxicity. The use of chlorpyrifos to area of fish breeding causes toxicological hazards especially in view of its high toxicity to fish (HERZBERG, 1987).

Fish are exposed to aquatic toxicants via the extensive and delicate respiratory surface of the gills and, in sea water, also via drinking (WENDELAAR BONGA, 1997). They are also exposed to these toxicants through feeding. The gill is a multifunctional and complex organ with which fish make intimate contact with the surrounding water. The gills comprise over half the body surface area and only a few microns of delicate gill epithelium separate the internal environment from a continually flowing external environment (WOOD; SOIVIO, 1991). Thus branchial function is very sensitive to environmental toxicants. Hence, for the study of toxicological impacts, fish serves as an excellent bioassay animal and have been widely used for this purpose (BLAXTER; HALLERS-TJABBES, 1992; ESPELID et al., 1996; HOLLIS et al., 1999; KUMAR et al., 2007; PRATAP, 1999; RUGGIERI, 1975; SWARUP et al., 1977; WENDELAAR BONGA, 1997).

Prolactin has a physiological role in certain biological features among higher vertebrates such as stimulation of the pegion crop, stimulation of mammary gland and post-ovulatory corpus luteum in some mammals and induction of water-drive and land-water integumentary changes in the urodeles (MEITES; NICOLL, 1966; VELLANO et al., 1967). However, fish prolactin does not elicit these responses. Prolactin has been considered as hypercalcemic factor in teleosts (FARGHER; McKEOWN, 1989; FLIK et al., 1994; PANG, 1981; SRIVASTAV; SWARUP, 1985; SRIVASTAV et al., 1995a, b; WENDELAAR BONGA; FLIK, 1982; WENDELAAR BONGA et al., 1985). Recently, a role of prolactin in embryonic-stage organogenesis in zebrafish (NGUYEN et al., 2008) and in the nuptial colouration in female fish has been reported (SKOLD et al., 2008). Although, several authors have reported the effects of organophosphate pesticides in fishes (AGBONE et al., 2002; JOHAL et al., 2007; ORUC et al., 2006; PATIL; DAVID, 2008; TILAK; SWARNA KUMARI, 2009; ZHANG et al., 2009), but there exist a single report regarding the effect of these organophosphates on the histological structure of prolactin cells of fish (MISHRA et al., 2008). Thus in the foregoing study we have made an effort to explore further the effects of organophosphate namely chlorpyrifos on the prolactin cells of a fish, *Heteropneustes fossilis*.

Material and methods

Live specimens of adult freshwater catfish, *Heteropneustes fossilis* (both sexes, body weight 42-53 g; body length 16-20 cm) were collected locally (from Ramgarh lake through a commercial supplier) and acclimatized to the laboratory conditions for 15 days in plastic tanks (50 fishes in each; dimensions 48 x 40 x 22 inch; capacity 125 gallon). The physicochemical characteristics of the tap water were used in the experiment are - photoperiod 11.58-12.38h; temperature 25.80 ± 1.80°C; pH 7.21 ± 0.06; hardness 167.32 ± 5.81 mg L^{-1} as $CaCO_3$; electrical conductivity 306.18 ± 68.52 µmhos cm^{-1}; dissolved oxygen 7.78 ± 0.30 mg L^{-1} and no free chlorine. During acclimatization the fish were fed daily with wheat flour pellets and ground dried shrimps, 2-3 times per day. Water was renewed daily after cleaning the fecal matter and left overfood. The fish were not fed 24h before and during the experimental period (short- and long-term).

In the present study, an organophosphorus compound Coroban (active ingredient chlorpyrifos–E.C. 20% manufactured by Coromandel Indag Products, Madras) was used. In short-term exposure the fish were subjected to 0.8 of 96h LC_{50} value of chlorpyrifos (1.76 mg L^{-1}) for 96h. In long-term exposure the experiment was performed for 28 days by using 0.2 of 96h LC_{50} value of chlorpyrifos (0.44 mg L^{-1}). Simultaneously, a control group was also used for comparison by using the tap water containing acetone (the volume of acetone used was the same as used for preparing the pesticide solution at different concentration). Fish were kept in groups of 10 in 40 L media (tap water containing acetone for control and pesticide's solution prepared in acetone for experimental). Six fish were killed on each time intervals from control and experimental (chlorpyrifos) groups after 24, 48, 72, and 96h in short-term exposure and after 7, 14, 21, and 28 days in long-term experiment.

Blood samples (approx 1 mL) were collected by sectioning the caudal peduncle. The sera were separated by centrifugation (3000 r.p.m.) and analyzed for calcium (TRINDER, 1960). After collection of blood samples, pituitary glands along with the brain were fixed in aqueous Bouin's fluid and Bouin's-Hollande fixatives for histological studies. Tissues were routinely processed in graded series of alcohols, cleared in xylene and embedded in paraffin wax. Serial sections were cut at 6 µm. The pituitaries were stained with Herlant tetrachrome and Heidenhain's azan techniques.

Nuclear indices (maximal length and maximal width) of the prolactin cells (50 nuclei from each fish, thus 300 nuclei from 6 fish) were taken with

the aid of ocular micrometer and the nuclear volume was calculated as:

$$volume = 4/3\ \pi\ ab^2$$

where:

'a' is the major semiaxis; and

'b' is the minor semiaxis. Student's t test was used to test for significant differences between the control and experimental groups with $p < 0.05$ being accepted as significant.

Results and discussion

The serum calcium levels of *H. fossils* exhibit a decline after 24h following exposure to chlorpyrifos. This decrease continues till the end of the experiment (96h; Figure 1). The serum calcium levels of chronically-exposed fish exhibit a decrease on day 7. Thereafter, the levels continue to fall progressively till the end of the experiment (28 days; Figure 2).

In control fish, the prolactin cells are predominant cell type of rostral pars distalis. The cell boundaries of these cells are indistinct (Figure 3). However, the nuclei are oval to round in shape with dense chromatin granules.

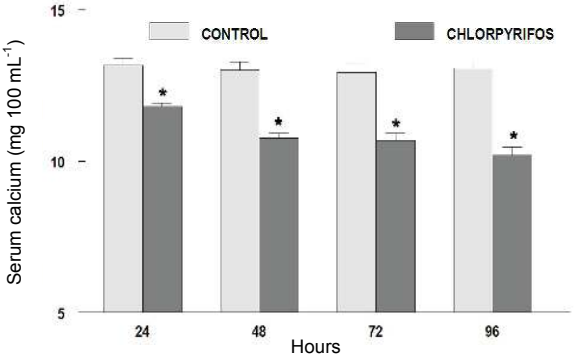

Figure 1. Serum calcium levels of short-term chlorpyrifos treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($p < 0.05$) from control.

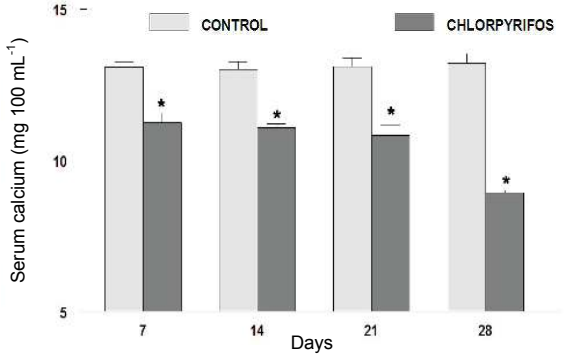

Figure 2. Serum calcium levels of long-term chlorpyrifos treated *Heteropneustes fossilis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences ($p < 0.05$) from control.

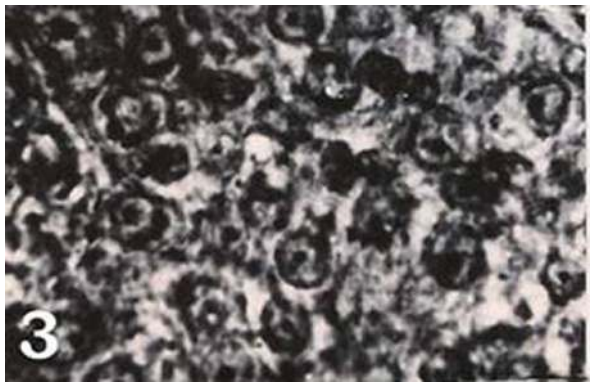

Figure 3. Prolactin cells of control *Heteropneustes fossilis*. Mark indistinct cell boundaries. Herlant tetrachrome X 800.

The prolactin cells of *H. fossilis* exposed to chlorpyrifos exhibit no change in the histological structure throughout the short-term experimentation whereas in long-term treated fish, the prolactin cells show no histological change up to 14 days. After 21 days, these cells exhibit slight degranulation (Figure 4). Following 28 days chlorpyrifos exposure, the prolactin cells exhibit further degranulation and at some places, vacuolization and cytolysis are also observed (Figure 5). The nuclear volume records an increase (Figure 6).

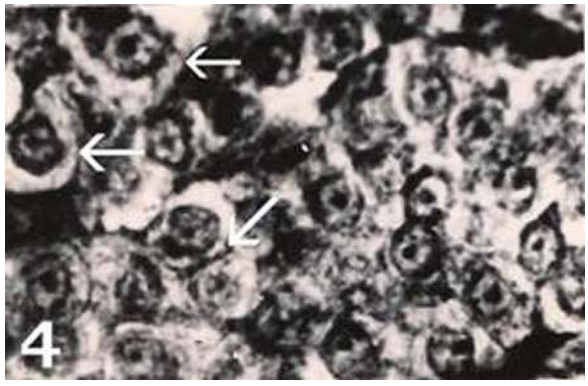

Figure 4. Prolactin cells of 21 days chlorpyrifos treated fish showing slight degranulation (arrows). Herlant tetrachrome X 800.

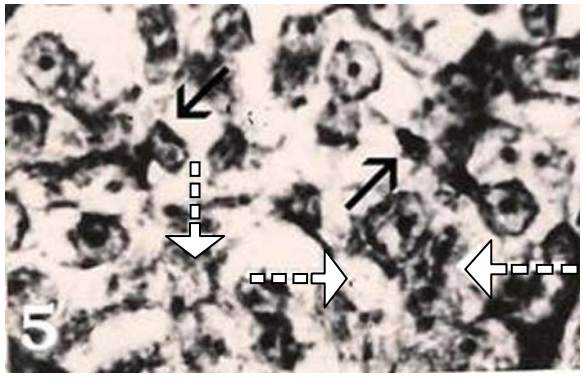

Figure 5. Prolactin cells of 28 days chlorpyrifos exposed fish showing degeneration (arrows). Note vacuolization and cytolysis (white broken arrows) at some places. Herlant tetrachrome X 800.

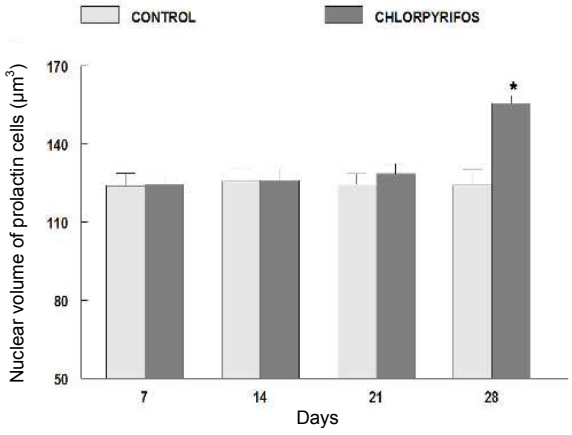

Figure 6. Nuclear volume of prolactin cells of long-term chlorpyrifos exposed *Heteropneustes fossilis*. Each value represents mean ± S.E. of six specimens. Asterisk indicates significant differences ($p < 0.05$) from control.

In the present study chlorpyrifos exposure caused hypocalcemia in fish *H. fossilis*. This is in agreement with reports of earlier investigators who have observed decreased blood/plasma content of calcium of fish treated with toxicants – aldrin (BANO, 1982; SINGH et al., 1996), malachite green (SRIVASTAVA et al., 1995b), cadmium (GILES, 1984; KUROSHIMA, 1987; LARSSON et al., 1981; MURAMOTO, 1981; PRATAP et al., 1989), propoxur (SINGH et al., 1997) and formothion (SINGH et al., 1997). In contrast, other investigators have observed elevation of plasma calcium levels of fish exposed to pesticides (BANSAL et al., 1979; DALELA et al., 1981; SASTRY; SHARMA, 1978; SHARMA et al., 1982). However, no statistically significant effect on blood/plasma calcium concentrations has been observed in DDT treated flounders *Platichthys flesus* (HAUX, 1979), methoxychlor treated Northern puffer *Sphaeroides maculatus* (EISLER, 1967) and bifenthrin treated rainbow trout, *Oncorhynchus mykiss* (VELISEK et al., 2009).

Prolactin cells of *H. fossilis* exhibit degranulation and increased nuclear volume after chlorpyrifos treatment. The activity of the prolactin cells has been judged by the staining response and nuclear volume. In past, the increased nuclear size/area/volume has been considered as indications for the hyperactivity of various endocrine glands by several investigators (OLIVEREAU; OLIVEREAU, 1982; OLIVEREAU et al., 1986; SRIVASTAV; RANI, 1992a and b). To the best of our knowledge the effect of organophosphate on prolactin cell activity has been studied only by Mishra et al. (2008). The present study is in conformity with studies of Mishra et al. (2008) as they have reported hyperactivity of prolactin cells of catfish after metacid-50 treatment (an organophosphate). The effect of toxicants on the prolactin cell activity has also been reported after cadmium (JAMES; WIGHAM, 1986) and cypermethrin (MISHRA et al., 2011). The result of Mishra et al. (2011) strengthen this study as they noticed an increased activity of prolactin cells in cypermethrin-treated catfish. The present study derives support from the studies of Thangavel et al. (2005) and Meredith et al. (1999) who have reported elevated levels of prolactin in fish treated with dimecron and DDT, respectively. The observed hyperactivity in the prolactin cells of chlorpyrifos exposed *H. fossilis* may be attributed to the hypocalcemia noticed in the present study after pesticide treatment as prolactin provoke hypercalcemia in various species of fishes(FLIK et al., 1986, 1994; PANG et al., 1978; WENDELAAR BONGA; FLIK, 1982; WENDELAAR BONGA; PANG, 1991).

Conclusion

It can be concluded from the present study that chlorpyrifos provoked hypocalcemia in the freshwater catfish *H. fossilis* which caused the hyperactivity of prolactin cells. In fishes calcium has been reported to play an important role in growth, reproduction, and several other physiological functions such as muscle contraction, nerve conduction, membrane permeability etc. (WENDELAAR BONGA; FLIK, 1993). Also calcium has been considered as having a direct stimulating effect on sperm motility (AMIN, 1998; BAYNES et al., 1981). In this study we have noticed continued hypocalcemia after chlorpyrifos exposure to the fish, hence it can be concluded that exposed fish may have disturbed physiological functions including reproduction and sperm motility.

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