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Prolactin cells of a teleost, *Heteropneustes fossilis*, intoxicated with Metacid-50

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ABSTRACT

The fish *Heteropneustes fossilis* were subjected to 5.28 mg/L (80% of 96 h LC_{50}) and 1.32 mg/L (20% of 96 h LC_{50}) solution of Metacid-50 (active ingredient methyl-parathion) for short-term (96 h) and long-term (28 days), respectively. *H. fossilis* exposed for short-term (96 h) to sublethal concentration of Metacid-50 exhibited a marked decrease in plasma calcium level. In short-term experiment, no change was noticed throughout the experiment in the histological structure and nuclear volume of prolactin cells of Metacid-50 treated fish. The chronical exposure of fish for long-term to Metacid-50-provoked hypocalcaemia. Up to 14 days there was no histological change in the prolactin cells of Metacid-50-exposed fish. After 21 days these cells exhibited slight degranulation. However, the nuclear volume remained unchanged. The prolactin cells exhibited further degranulation and the nuclear volume recorded an increase following 28 days Metacid-50 exposure. At some places, vacuolization and cytolysis were also observed.

Keywords: Prolactin cells, Organophosphate, catfish, plasma calcium, Metacid-50; methyl-parathion.

INTRODUCTION

Among higher vertebrates prolactin has a physiological role in certain biological features such as stimulation of the pigeon 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. However, fish prolactin does not elicit these responses. Several investigators have reported that prolactin is a hypercalcaemic factor in fishes (Pang, 1981; Wendelaar Bonga and Flik, 1982; Wendelaar Bonga et al., 1985; Srivastav and Swarup, 1985; Fargher and McKeown, 1989; Flik et al., 1994; Srivastav et al., 1995). Recently, Nguven et al. (2008) have reported a role of prolactin in embryonic-stage organogenesis in zebrafish. A role of prolactin in the nuptial coloration in female fish has been reported by Skold et al. (2008).

Pesticides have unique position among the chemicals as they are deliberately added to the environment for the purpose of killing some life forms. Ideally their effect would be highly specific for undesirable target organisms and harmless to desirable non-target organisms. Most of the chemicals used as pesticides are not highly selective but are generally toxic to other species, including man, and other desirable forms of life that shares the environment.

An organophosphate insecticide Metacid-50 (O, O-dimethyl O-P-nitrophenyl phosphorothioate), is an effective contact and stomach poison which is increasingly applied for controlling pests of cotton, sugarcane, tobacco, paddy, cucurbits, fruit trees, potatoes, citrus and coffee, and controls variety of insects such as aphids, mites, beetles, lepidoptera, leaf hoppers, leaf miners and several soil insects (Greene et al., 2001; Fragoso et al., 2002; Wakgari and Giliomee, 2003). Although, several workers have reported the toxicity of pesticides in fishes, there exist few reports regarding their effect on blood electrolytes of fish (Srivastav et al., 1997, 2008; Singh et al., 1997; Mishra et al.,

2005). Moreover, elevated levels of plasma prolactin in fish have been reported after exposure to dimecron (Thangavel et al., 2005) and DDT (Meredith et al., 1999). Plasma prolactin concentrations were transiently depressed after 12 hr in trout exposed to sublethal aluminium in acidic soft water (Waring et al., 1996). There exists no published report regarding the effects of organophosphate on histological structure of prolactin cells of pituitary gland in fish. Thus we aimed to study such effect of an organophosphate, Metacid-50 (active ingredient methyl-parathion), on prolactin cells of a fish, *Heteropneustes fossilis*.

MATERIALS AND METHODS

Live specimens of adult *H. fossilis* (both sexes, body wt. 32-44 g) were procured locally and acclimatized to the laboratory conditions (under natural photoperiod 11.28-12.06 and temperature 26.72 ± 2.14 °C) for 15 days in plastic pools. Each pool contained 500 L of dechlorinated tap water. The physicochemical conditions of the tap water used in experiment were temperature 26.72 ± 2.14 °C, pH 7.28 ± 0.08, hardness 169.37 ± 5.78 mg/L as $CaCO_3$, dissolved oxygen 7.87 ± 0.34 mg/L, electrical conductivity 307.16 ± 65.12 µmho/cm. 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 leftover food. Care was taken to avoid giving stress to the fish. The mortality rate during the acclimatization was less than 4 %.

After acclimatization, the fish *H. fossilis* were subjected to 5.28 mg/L (80% of 96 h LC_{50}) and 1.32 mg/L (20% of 96 h LC_{50}) solution of Metacid-50 (active ingredient methyl-parathion) for short-term (96 h) and long-term (28 days) duration, respectively. A control group was also run concurrently. The media (both control and experimental) were renewed every 24 h. Food was withheld 24 h prior to the start of the experiment. The fish were sacrificed at 24, 48, 72 and 96 h in short-term experiment and at 7, 14, 21 and 28 days in long-term experiment. The blood was collected in heparinized tubes after sectioning of the caudal peduncle. Plasma calcium levels were determined by Sigma kit. The pituitary gland along with the brain was fixed in aqueous Bouin's fluid and Bouin's-Hollande fixatives. Tissues thus fixed were routinely processed in graded series of alcohols, cleared in xylene and then embedded in paraffin. Serial sections were cut at 6 µm and stained with Herlant tetrachrome and Heidenhain's azan techniques.

The nuclear indices (maximal length and maximal width) of prolactin cells were determined (fifty nuclei were measured per specimen, thus 300 nuclei were measured from six specimens) with the aid of an ocular micrometer and then the nuclear volume was calculated as:

Volume = $4/3\ \pi\ ab^2$; where 'a' is the major semi-axis and 'b' is the minor semi-axis.

Student's t test was used to test for significant differences between the control and Metacid-50 treated fish. A P value of <0.05 was considered statistically significant.

RESULTS

After short-term methyl-parathion exposure, the plasma calcium levels exhibited a decline after 24 h ($P<0.05$). This decrease continued ($P<0.05$) till the end of the experiment (96 h) (Figure 1). The chronically exposed fish to methyl-parathion exhibited a decrease in the plasma calcium levels at day 7 ($P<0.05$). Thereafter, the levels progressively decreased ($P<0.05$) till the end of the experiment (28 days; Figure 2).

In control fish, the prolactin cells possessed indistinct cell boundaries (Figure 3). However, the nuclei were distinct - with dense chromatin granules. The cytoplasm was scanty and was azocarminophilic and erythrosinophilic in nature.

In short-term experiment, methyl-parathion produced no change in the histological structure and nuclear volume of prolactin cells of *H. fossilis*. In chronically exposed fish, there was no histological change in the prolactin cells up to 14 days. After 21 days these cells exhibited slight degranulation (Figure 4). However, the nuclear volume remained unchanged (Figure 5). The prolactin cells exhibited further degranulation (Figure 6) and the nuclear volume recorded an increase ($P<0.05$) following 28 days methyl-parathion exposure (Figure 5). At some places, vacuolization and cytolysis were also observed (Figure 7).

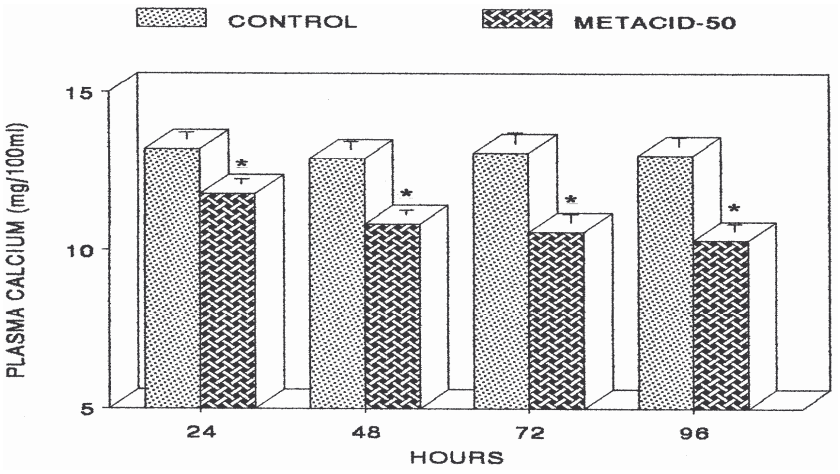

Figure 1: Comparisons between plasma calcium levels of control and short-term Metacid-50-treated *Heteropneustes fossilis*. Values are Mean ± SEM of six specimens. Asterisk indicates significant differences (P< 0.05) from control group.

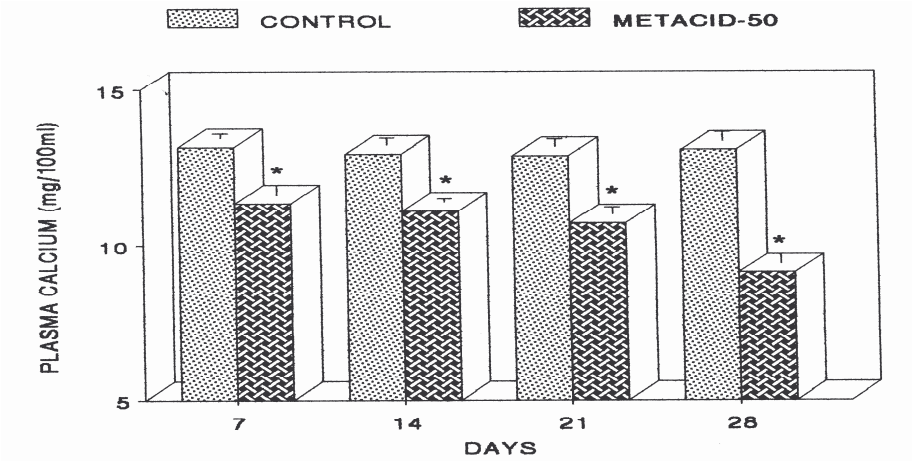

Figure 2: Comparisons between plasma calcium levels of control and long-term Metacid-50-treated *Heteropneustes fossilis*. Values are Mean ± SEM of six specimens. Asterisk indicates significant differences (P< 0.05) from control group.

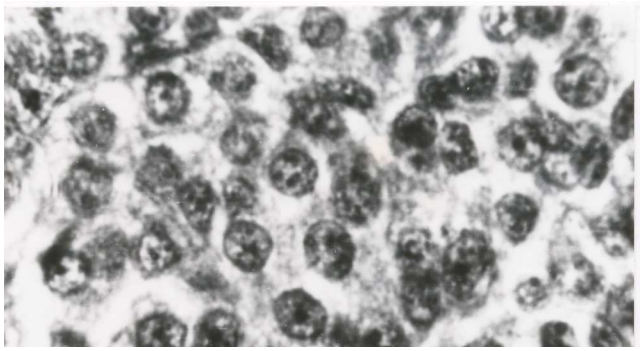

Figure 3: Prolactin cells of control *Heteropneustes fossilis*. Herlant tetrachrome X 800.

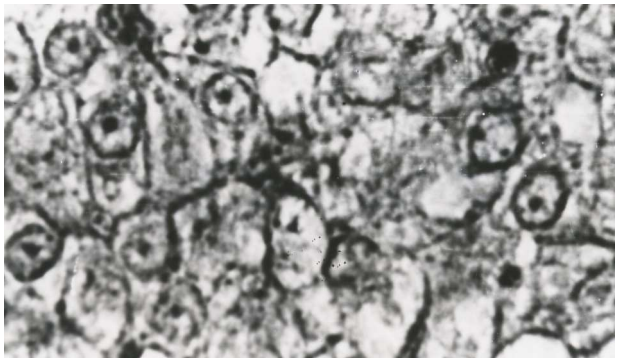

Figure 4: Prolactin cells of 21 days Metacid-50-treated fish showing slight degranulation. Herlant tetrachrome X 800.

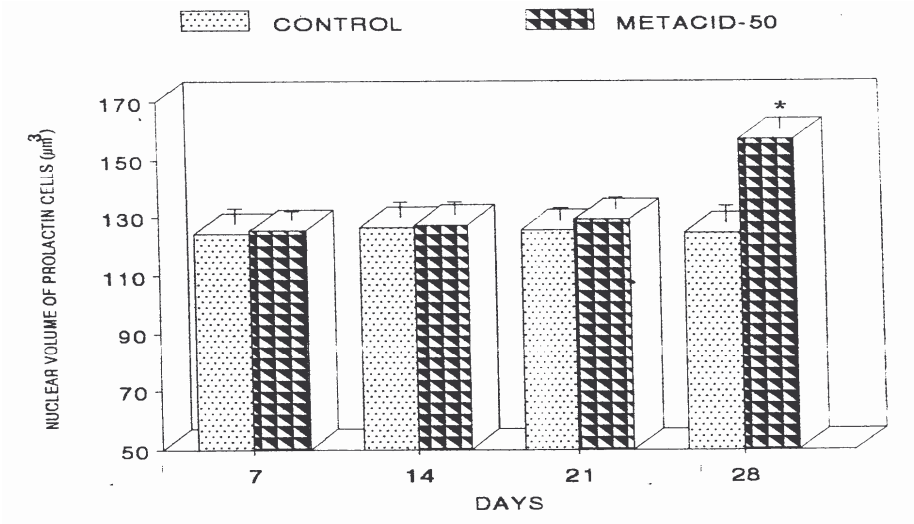

Figure 5: Nuclear volume of prolactin cells of long-term Metacid-50-exposed *Heteropneustes fossilis*. Each value represents Mean ± SEM of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

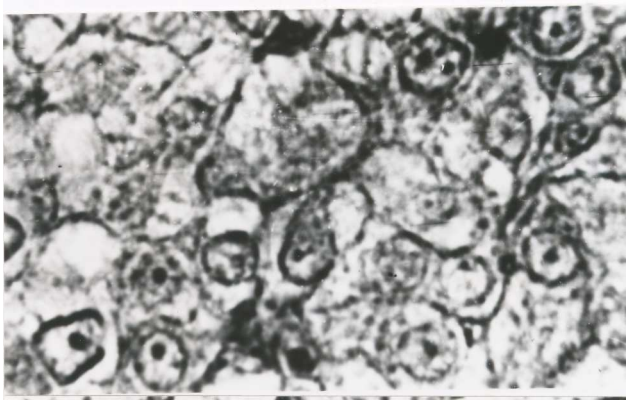

Figure 6: Prolactin cells of 28 days Metacid-50-treated fish exhibiting degranulation. Herlant tetrachrome X 800.

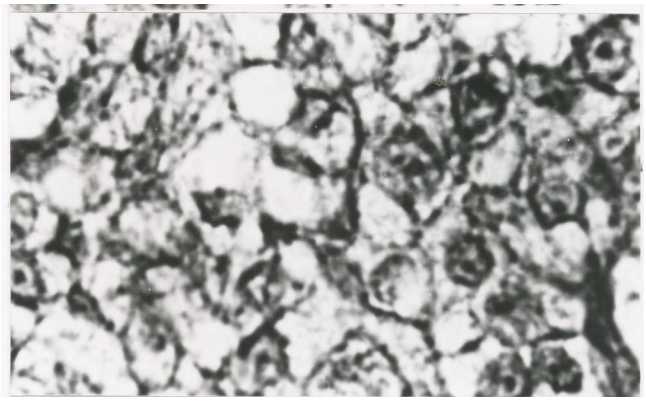

Figure 7: Prolactin cells of 28 days Metacid-50-exposed *Heteropneustes fossilis* showing vacuolization and cytolysis. Herlant tetrachrome X 800.

DISCUSSION

Exposure of fish *H. fossilis* to Metacid-50 caused a decrease in the plasma calcium level. This supports the observations of earlier investigators who have reported decreased blood/plasma calcium content of fish treated with toxicants - deltamethrin (Srivastav et al., 1997), cypermethrin (Mishra et al., 2005), aldrin (Singh et al., 1996), malachite green (Srivastava et al., 1995), cadmium (Rai and Srivastav, 2003), propoxur (Singh et al., 1997) and formothion (Singh et al., 1997). Contrary to this, an elevation of plasma calcium levels of fish exposed to pesticides has been noticed by Bansal et al. (1979), Dalela et al. (1981) and Sharma et al. (1982). However, Haux (1979) observed no effect on blood/plasma calcium concentrations of DDT-treated flounders *Platichthys flesus*.

Hyperactivity of prolactin cells has been observed in fish exposed to methyl-parathion for long durations. This response is evident by degranulation and increased nuclear volume of prolactin cells. The present study derives support from the studies of Thangavel et al. (2005) and Meredith et al. (1999) who have also reported elevated levels of plasma prolactin in fish treated with dimecron and DDT, respectively. It gets further support from Fu (1989) who observed an increased activity of prolactin cells in cadmium exposed tilapia. However, James and Wigham (1986) did not find any consistent effect on prolactin cell activity in cadmium injected rainbow trout. Prolactin has been reported to provoke hypercalcaemia in various species of fishes (Wendelaar Bonga and Flik, 1982; Flik et al., 1994; Wendelaar Bonga and Pang, 1991) mainly by controlling the gill epithelium permeability (Dharmamba and Meatz, 1972; Wendelaar Bonga et al., 1983). The observed hyperactivity of the prolactin cells may be attributed to the hypocalcaemia noticed in the present study after methyl-parathion treatment. Prolactin may also exert its hypercalcaemic action *via* mobilization of calcium from exchangeable calcium stores. Bone demineralization has been reported in cadmium-exposed carp (Koyama and Itazawa, 1977; Muramoto, 1981) and has been interpreted as a mechanism to restore plasma calcium levels. The present study lacks evidence in support of a similar action in methyl-parathion-treated catfish since the calcium content of exchangeable calcium stores was not measured.

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