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Dimethoate induced changes in serum Ca^{2+} and Corpuscles of Stannius in freshwater catfish *Heteropneustes fossilis*, after short-term and long-term exposure

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Abstract: The Corpuscles of Stannius (CS) exclusively present in bony fishes, produce Stanniocalcin – an anti-hypercalcemic hormone. Fish are in intimate contact with their surrounding through their gills. Gills are the main target of stanniocalcin; therefore, the activity of CS is influenced by many external and internal factors. Stressful conditions such as exposure to various pesticides disturb the ionic balance in fishes. Short-term (96 h) and long-term (36 d) toxic effect of an organophosphate insecticide, dimethoate was studied on serum Ca^{2+} and CS histology in freshwater air-breathing catfish *Heteropneustes fossilis*. The concentration of dimethoate for short-term exposure was 2.24 mg L^{-1} (75% of 96 h LC_{50}) and for long-term was 0.75 mg L^{-1} (25% of 96 h LC_{50}). Fish exhibited hypocalcaemia in both the short-term as well as in long-term experiment. The glandular cells of CS in short-term experiment, exhibited increase in the nuclear volume (NV) along with increase in the nucleoli number after 24 h and thereafter, show decrease in NV and deformity of nucleus after 96 h dimethoate exposure. In long-term experiment, the NV increased following 6 d of exposure to dimethoate, but decreased significantly thereafter. The gland exhibited degeneration after 36 d of dimethoate exposure.

Keywords: Dimethoate, Calcium, Corpuscles of Stannius, *Heteropneustes fossilis*

1. Introduction

The Corpuscles of Stannius (CS) are unique glands present in the kidney of bony fish, producing an anti-hypercalcemic hormone - Stanniocalcin [1]. The cells of corpuscles of Stannius in fish possess extracellular calcium sensing receptors [2] and help in calcium uptake from gill epithelium [3]. Fish are in intimate contact with their environment through gills; therefore, activity of CS is influenced by several external and internal factors. Environmental contaminants adversely affect the gills [4-6] and therefore, disrupt entire mechanism of electrolyte regulation in fish [7-11]. Exposure of pesticides adversely affects the function of CS and calcium homeostasis in fish [12-14].

Dimethoate a wide spectrum systemic and contact OP insecticide with 68 days half life at 25 ± 1^0C and pH 7 has been reported extremely toxic to fish and other beneficial arthropods and mollusks [15]. To the best of our knowledge there is no information regarding impact of dimethoate on histology of CS. Therefore, we aimed to study its effect on serum Ca^{2+} and CS of *Heteropneustes fossilis.*

2. Materials and Methods

The adult catfish (length: 17.4 ± 1.1 cm and weight: 27.1 ± 2.0 g; both sexes) *Heteropneustes fossilis*, were procured from local ponds and carefully brought to the laboratory, avoiding any injury during transportation. Fish were treated with 0.05% $KMnO_4$ solution for 2 minutes and kept in plastic tank (500 L) to acclimatize under laboratory conditions for 14 days. The experiment was conducted during the month of October. The physicochemical properties of test water were - temperature (23 ± 1 °C) pH (7.2 ± 0.15), dissolved oxygen (7.9 ± 0.57 mg L^{-1}) and hardness as $CaCO_3$ (118.4 ± 1.25 mg L^{-1}). Fish were daily fed with a mixture (about 0.1 gm/ fish) of wheat flour, mustard cake, dried prawn powder, and

soybean in a ratio of 3:1:1:1. No mortality was recorded in control and experimental group. Dimethoate 30% Effective Concentration technical grade (Rallis India Pvt. Ltd., Mumbai, India) was used. Glass aquaria (30 L capacity) filled with 25 L tap water, were used for the experiments.

The LC_{50} of dimethoate in fish had been determined (3.0 mg L^{-1} for 96 h) earlier using probit analysis software [16]. Therefore, the sub-lethal concentration of dimethoate was taken as 2.25 mg L^{-1} (75% of 96 h LC_{50}) for short-term exposure and 0.75 mg L^{-1} (25% of 96 h LC_{50}) for long-term exposure. Fish were not fed 24 h before and during the experiment. The experiment was carried out to study short-term (96 h) and long-term (36 d) sub-lethal effect of dimethoate on serum calcium levels and histological changes in CS gland. The design and basic procedure for short-term and long-term study were kept same. In short-term experiment 80 acclimatized fish were separated in two equal groups: control and test (dimethoate exposed). Similarly for long-term 80 fish were separated in two equal groups – control and dimethoate exposed test group. 10 fish were sacrificed from each group (control, test) on each interval after 24, 48, 72, and 96 h (short-term) and 6, 12, 24, and 36 d (long-term) experiment. Thus, a total of 160 fish were used during the experiment. Blood samples were collected on above mentioned intervals in citrated tuberculin syringes directly from the conus arteriosus under anesthesia with tricaine methane sulfonate (1g/3L). At the same time CS gland together with adjoining portions of kidney was also extricated and fixed in Bouin's fluid *in situ* for 24 h. After which the tissue was washed with water and dehydrated in graded series of alcohols, cleared in xylene before embedding in paraffin wax. Serial sections of 5-6 µm were cut, stained in Haematoxylin - Eosin and mounted in DPX. To determine the average nuclear diameter of the gland cells, 50 nuclei were randomly selected from every fourth section of the gland and their diameter was measured with the help of oculometer under oil immersion (x1000). In total over 200 nuclei were always measured for each gland. The nuclear volume (NV) was calculated by the formula - $NV = 4/3\ \pi\ a.b^2$, where, 'a' is the major nuclear axis and 'b' represents the minor nuclear axis.

Serum Ca^{2+} levels were measured using Erba Mannheim diagnostic kit (manufactured by Erba diagnostics Mannheim GmbH, Germany). The serum Ca^{2+} levels and NV of CS cells (control and exposed fish) was statistically evaluated by Student's t-test (two tailed) for significance ($P < 0.05$ being accepted as significant).

3. Results

After short-term dimethoate (75% of 96 h LC_{50} = 2.24 mg L^{-1}) treatment, the serum Ca^{2+} levels of fish *H. fossilis* recorded significant (P<0.05) increase following 24 h exposure. The calcium levels, however, showed a decline after 48 h and continued to fall gradually up to 96 h, exhibiting significant (P<0.0001) hypocalcaemia in the exposed fishes (Fig.1). The serum Ca^{2+} level in long-term dimethoate exposure (0.75 mg L^{-1}= 25% of 96 h LC_{50}), exhibited a minor increase after 6 days, thereafter decreased gradually up to 36 d, showing significant (P<0.0001) hypocalcaemia (Fig.1).

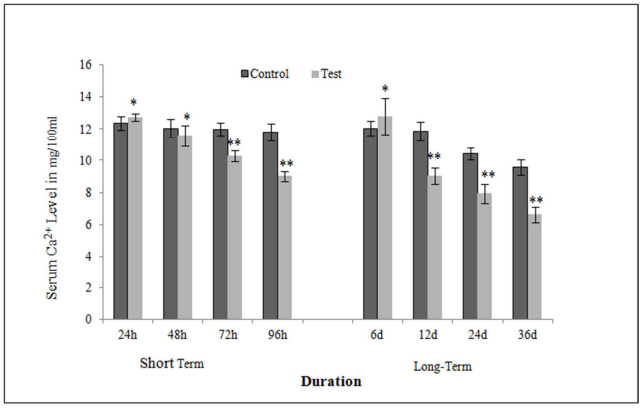

Figure 1. *Serum Ca^{2+} level in Heteropneustes fossilis after short term (75% of 96 h LC_{50} = 2.24 mg L^{-1}) and long term (25% of 96 h LC_{50} = 0.75 mg L^{-1}) dimethoate exposure. Values are given as Mean ± SD, n=10 (Students 't-test, Significant values: * P<0.05, ** P<0.001).*

Histological examination reveals that CS are embedded in the renal tissue and its lobules or cell cords (CSL) are separated by connective tissue enclosing rich capillary network (CN). In control, CS exhibit normal cells in lobules with rounded nuclei (N) (Fig.3).

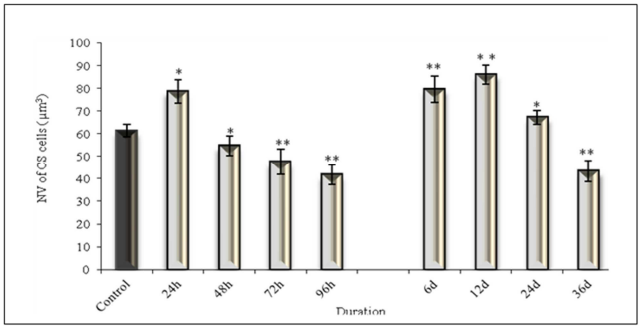

Figure 2. *Nuclear Volume (NV) of CS cells (Values are given as Mean ± SD, n=250) after dimethoate exposure in Heteropneustes fossilis (Students 't-test, Significant values: * P<0.05, ** P<0.001*

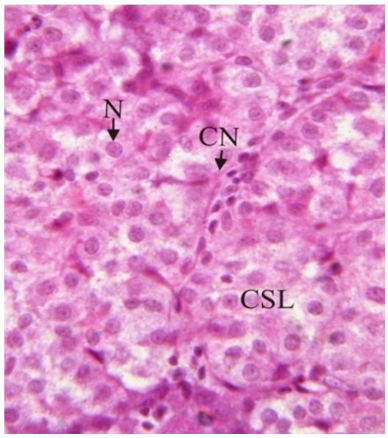

Figure 3. *CS control showing the lobules (CSL), capillary network (CN); lobular cells possessing rounded nuclei (N) in the center [H/E: X1000]*

In short-term experiment, after 24 h dimethoate exposure, the glandular cells of CS exhibited hyperactivity marked by increase in nuclear volume (NV) and nucleoli number, dilation in lobules and central space of lobules filled with debris (D) and basophilic material (Fig.2, 4). The NV recorded a decrease after 48 h which continued up to 96 h (Fig.2). The CS of 96 h exposed fishes showed glandular disintegration, majority of cells exhibited nuclear deformity and shrinkage (N), the blood capillaries (CN) within gland became more dilated and the central space of cell cord has been occupied with degenerating cells (D) (Fig.5).

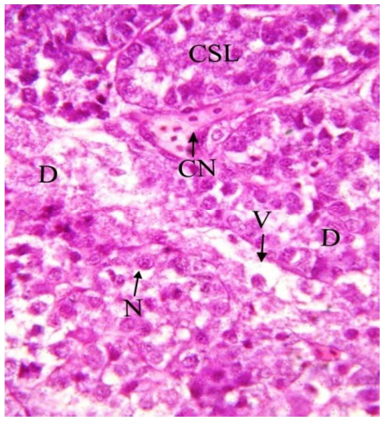

Figure 4. *24 h dimethoate treated CS exhibiting hyperactivity; increase in nuclear volume (N), number of nucleoli and the number of cells in dilated lobules (CSL); the central space of the lobules filled with cellular debris (D) and the basophilic material; dilated capillary network (CN). [H/E: X1000]*

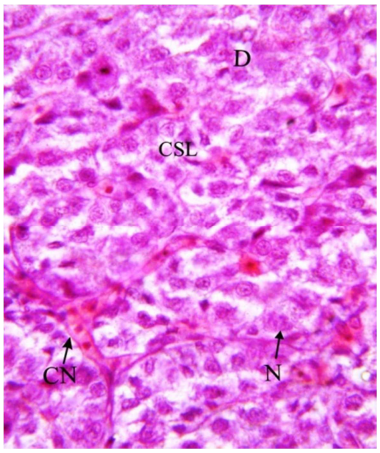

Figure 5. *96 h dimethoate treated CS showing disintegration of lobule (CSL); nuclear deformity and shrinkage in majority of cells (N); degenerating cells in central space (DC); vacuolated central space with cell debris (D); dilated capillary network (CN). [H/E: X1000]*

In long-term experiment the CS showed hyperactivity recorded by increase in nuclear volume of the cells up to 12 d of dimethoate exposure (Fig.2). The CS exhibited cellular degeneration and vacuolization (D), and dilation in lobules (CSL) and blood capillaries after 6d (Fig.6). After 24 d treatment, the gland showed significant decrease in nuclear volume, nuclear degeneration and shrinkage (DN) and lobular degeneration (DL) (Fig.2, 7). The gland exhibited further degeneration and shrinkage in the nucleus and prominent cytoplasmic vacuolization after 36 d of dimethoate exposure. The NV recorded a decrease with highly indented nucleus (Fig.8).

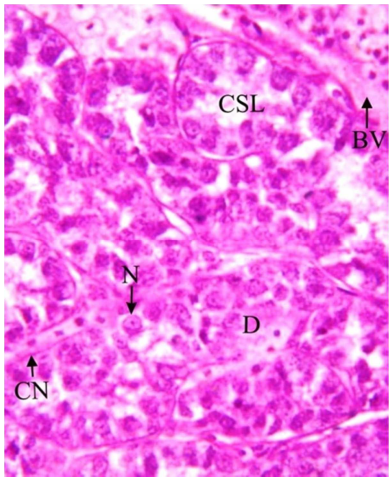

Figure 6. *6 d dimethoate treated CS exhibiting increased nuclear volume (N); dilated lobules (CSL) showing degeneration and vacuolization (D) and prominently dilated blood capillaries (CN). [H/E: X1000]*

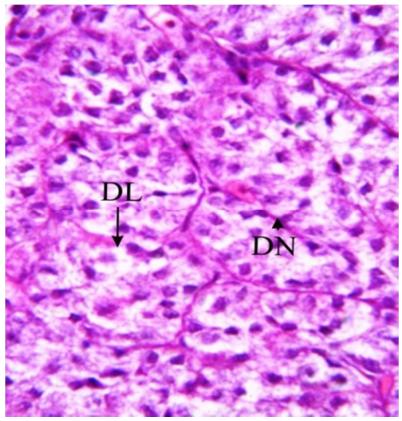

Figure 7. *24 d dimethoate treated CS exhibiting degeneration and shrinkage in the nuclei (DN); and degenerating CS lobes (DL). [H/E: X1000]*

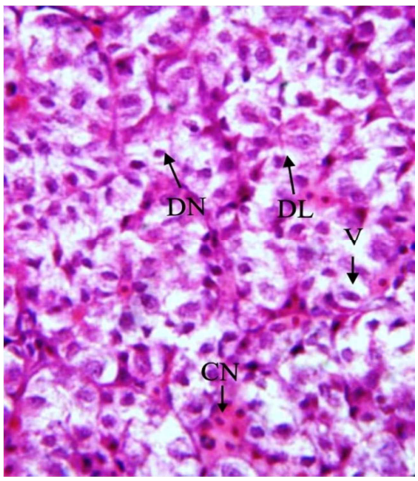

Figure 8. *36 d dimethoate treated CS exhibiting degeneration and shrinkage in the nuclei (DN); decreased nuclear volume (N); and degenerating CS lobes (DL); highly indented nuclei and prominent cytoplasmic vacuolization (V); dilated blood capillaries (CN). [H/E: X1000]*

4. Discussion

Dimethoate exposure induces hypocalcaemia after short-term (75% of 96 h LC_{50} = 2.24 mg L^{-1}) and long-term (25% of 96 h LC_{50} =0.75 mg L^{-1}) treatment in *Heteropneustes fossilis*. Initial increase (at 24 h and 6 d) in calcium level (Fig.1) may be due to uptake of calcium from gills in order to maintain Ca^{2+} homeostasis and to support certain physiological activities. The decline in Ca^{2+} after 48 h and 12 d (Fig.1) may be an indication of exhaustion of the Ca^{2+} depots and its reduced uptake from gills and kidney tissues due to damage induced by the pesticide. Degenerative changes in gills after pesticide exposure lead to reduced ionic influx and decreased levels of ions in blood [17-18]. Hypocalcaemia in dimethoate exposed fish may also be due to degenerative changes in kidney [4, 18-19]. Tubular necrosis leads to decreased Ca^{2+} ion reabsorption and enhanced urinary excretion in dimethoate exposed catfish. Hypocalcaemia exhibited by the fish during short-term and long-term exposure to dimethoate is similar to the observations of earlier workers with chlorpyrifos [14] formothion and propoxur [20], cypermethrin [7, 21], and deltamethrin [22] pesticides. Thangavel et al. however, recorded a decline in serum Ca^{2+} levels in a freshwater teleost, *Sarotherodon mossambicus* when exposed to dimecron [23]. A progressive decrease in serum calcium level was observed in *Heteropneustes fossilis* after 48 h (short term) and 7 d (long term) exposure to *Euphorbia royleana* latex [24]. Das et al. have reported hypocalcemia in *Heteropneustes fossilis* after dimethoate exposure [11].

The histological examinations revealed that CS exhibit alterations after short-term and long-term dimethoate exposure. Following 24 h of exposure, the glandular cells exhibit hyperactivity with significant (P<0.05, t- test) increase in nuclear volume (NV) and number of nucleoli (Fig.4). This stage corresponds to significant increase (P<0.05) in level of serum calcium probably due to influx of Ca^{2+} through gills. The observation was in contrast to the earlier reports depicting no change in the activity of CS cells following 24 h exposure to various pesticides [12-14, 25]. The NV however, recorded a decline after 48 h which continues to decline up t o 96 h (Fig.2). The CS of pesticide exposed fish exhibited cellular hyperplasia leading to cellular disintegration, vacuolization and nuclear deformity; these observations are in agreement with the findings of Srivastav et al. [22]. Similar results have been reported in CS of *Heteropneustes fossilis* exposed to a variety of pesticides [10, 12-14, 25].

After 6 d exposure to dimethoate, CS showed dilation in lobules (CSL) with degeneration and vacuolization (D), increase in nuclear volume and dilation in blood capillaries (CN). The nuclear volume increased up to 12 d and thereafter, declined steadily following 36 d exposure (Fig.2). The cells of the CS exhibited degeneration and shrinkage in nuclei (DN) and degeneration in CS lobe (DL) following 24 d exposure (Fig.7). After 36 d the gland exhibits more degeneration together with prominent cytoplasmic vacuolization in cell (Fig.8). This study derives support from the earlier works on CS of fish exposed to various harmful chemical substances [10, 13, 25]. Two cell types have been recognized in CS of *H. Fossilis* after Aldehyde fucshin staining: AF-positive cells (type -1 cells) and AF-negative cells [22]. AF-positive cells were accumulating AF-positive granules in fish exposed to low calcium / acalcic freshwater [26]. In present study hematoxylin/eosin stain has been used in which staining is inconspicuous and the major cell type - Type 1 [22], has been taken into account. It appears that CS has a single cell population with variable granulation: cells may be rich in AF granulation, then may be called – AF positive, otherwise AF –negative.

5. Conclusion

CS controls body calcium by secreting an anti-hypercalcemic hormone, the stanniocalcin. Therefore, initial increase in CS activity may be due to hypercalcaemia induced by dimethoate after 24 h and 6 d of exposure. Dimethoate exposure of fish for longer duration may however cause hypocalcaemia, which probably reduces the activity of CS. The prolonged hypocalcaemic condition is due to degeneration of gills and reduced Ca^{2+} influx.

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