Cyto-architectural Alterations in the Corpuscles of Stannius of Stinging Catfish Heteropneustes fossilis after Exposure to a Botanical Pesticide (Nerium indicum)

ManiRam Prasad[1], Abhishek Kumar[1], Sunil Kumar Srivastav[1], Nobuo Suzuki[2], Ajai Kumar Srivastav[1]*

Received: 28.04.2013 *Accepted: 01.06.2013*

ABSTRACT

Background: This investigation describes the cyto-architectural alterations observed in the corpuscles of Stannius of stinging catfish Heteropneustes fossilis after treatment with a botanical pesticide Nerium indicum.

Methods: Heteropneustes fossilis were subjected to 11.27 and 2.81 mg/L of Nerium indicum leaf extract over short- and long-term exposure periods, respectively. Blood was collected for calcium analysis and corpuscles of Stannius (CS) gland were fixed on 24, 48, 72 and 96 h in the short-term experiment and after 7, 14, 21, and 28 days in the long-term experiment.

Results: Serum calcium levels decreased from 48 h to 96 h. CS remains unaffected till 72 h. After the 96-hour treatment, increased granulation was observed in AF- positive cells. Nuclear volume of these cells exhibited no change throughout the short-term treatment. Slight increases in nuclear volume of AF-negative cells were recorded after 96 h. Nerium indicum caused decreases in serum calcium levels of H. fossilis from day 14 to 28. CS exhibited no alterations up to 14 days of exposure. AF-positive cells of CS depicted increased granulation after 21 days of treatment. Nuclear volume of these cells exhibited a slight decrease from day 21 to 28. Heavy accumulation of AF-positive granules was observed and few degenerating cells were noticed. Nuclear volume of AF-negative cells increased after 21 and 28 days of treatment. Vacuolization and degeneration occurred in certain places.

Conclusion: It is inferred from the present study that the botanical pesticide Nerium indicum induced severe changes in the corpuscles of Stannius of catfish.

Keywords: Botanical Pesticide, Calcium, Corpuscles of Stannius, Heteropneustes Fossilis, Nerium Indicum, Teleost.

IJT 2014; 1017-1024

INTRODUCTION

Botanical pesticides are phytochemicals that have evolved in plants for defense against phytophagous insects. Although derived from natural sources, botanicals are not necessarily safer or less toxic to non-pest insects, humans, and animals, than synthetically-derived pesticides. In fact, most botanicals cause allergic reactions in people, others are highly toxic to fish and animals, and some may even causes cancer. The use of plant species or their products (green pesticides or botanical pesticides) to control insect pests has been in practice to a limited extent for centuries. Recently interest has renewed in the pest management potential of these natural products. Natural pesticides based on plant extracts, such as rotenone, nicotine, and pyrethrum, have been commonly used for pest control during the earlier half of this century.

Nerium indicum is used as a medicinal plant in India. It is useful for the treatment of menorrhagia, asthma, bronchitis, and inflammation of gums [1]. Extract of herbal teas made of Neirum oleander has been ingested for suicidal or medicinal purposes

1. Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur, India.
2. PhD.Noto Marine Laboratory, Institute of Nature and Environmental Technology, Kanazawa University, Noto-cho, Ishikawa, Japan.
*Corresponding Author: E-mail: ajaiksrivastav@hotmail.com

[2,3] and as wood preservative [4]. Nerium is also used as rat poison and insecticide .

Corpuscles of Stannius (CS), which secrets an antihypercalcemic hormone Stanniocalcin, was considered to be unique to fish as they have not been identified in other vertebrates [5-8]. Fontaine [9] first established a correlation between CS and calcium regulation. Several studies have indicated the presence of two-cell types in CS [10-14]. On the other hand, other investigators have noticed only one-cell types in the CS of some teleost [15-17]. In non-piscine vertebrates, presence of functional receptors for STC have been suggested by few investigators on the basis of their studies in which they observed CS extract induced hypocalcemia in snakes [18] and in birds [19]. Stanniocalcin (STC) homologs may also be present in tetrapods [20]. In higher vertebrates, no homologous structure of CS has been present. However, immunocytochemically Stanniocalcin (STC 1 and STC 2) has been identified in the bladder, ovary, alpha cells of pancreas, and kidney of human and rat [20-22]. This study was an attempt to investigate the effects of Nerium indicum leaf extract on the corpuscles of Stannius of a teleost, H. fossilis. To the best of our knowledge, the effects of botanical pesticides on fish CS have not been reported yet.

MATERIALS AND METHODS

Adult stinging freshwater catfish Heteropneustes fossilis (both sexes bodyweight 32-44 g) were collected locally. Healthy fish showing no external signs of injury and disease were selected for experiments and acclimatized to laboratory conditions (under natural photoperiod 11.46-12.18 and temperature 26.74 ± 2.11 °C; pH 7.26 ± 0.09; hardness 167.97 ± 5.69 mg/L as CaCO3; dissolved oxygen 7.85 ± 0.36 mg/L) for 15 days in dechlorinated tap water. The Animal Research Ethical Committee of DDU Gorakhpur University, Gorakhpur, approved the study.

In the present study, Nerium indicum leaf extract was used. Prasad et al. [23] reported the 96-hour LC50 value of Nerium indicum leaf extract (14.0 mg/L for the fish H. fossilis). In short-term exposure, the fish were subjected to 11.27 mg/L of Nerium indicum leaf extract (80% of the 96-hour LC50 value). In long-term exposure, the fish were subjected to 2.81 mg/L (20% of 96 h LC50 value) of Nerium indicum leaf extract. At the same time, a control group was also run for comparison by using the tap water containing ethanol. Fish were kept in groups of 10 in 40 L media. Nerium indicum leaf extract was weight and stock solution (50 mg/ml) was prepared in 100% ethanol. Six fish were sacrificed (anaesthesized with MS 222) on each time interval from control and experimental (Nerium indicum) groups after 24, 48, 72, and 96 h in short-term exposure and after 7, 14, 21, and 28 days in long-term exposure.

Blood samples were collected by sectioning of the caudal peduncle of the fish. The sera were separated by centrifugation at 3500 rpm and analyzed for calcium levels (calcium kit, RFCL Limited, India). After collection of blood samples, the corpuscles of Stannius were fixed in aqueous Bouin's fluid. Fixed tissues were routinely processed in graded series of alcohols, cleared in xylene, and then embedded in paraffin wax. Serial sections were cut at 6 μm and stained with aldehyde fuchsin (AF) for light microscopic examination (Olympus CH 20i). Photomicrograph were taken with the aid of Olympus E 420 camera

Nuclear indices (maximal length and maximal width) of corpuscles of Stannius were determined (50 nuclei were measured per specimen; thus, 300 nuclei were measured from six specimens) with the aid of ocular micrometer and then the nuclear volume was calculated as

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

Where 'a' is the major semiaxis and 'b' is the minor semiaxis.

All data are presented as the mean ± S.E. of six specimens and student's t-test was used for the determination of statistical significance. In all studies, the experimental group was compared to its specific time control group. Two-way analysis of variance (ANOVA) was used for multiple group comparisons.

RESULTS

In the short-term experiment, the serum calcium levels of H. fossilis exhibits a decline after 48 h following exposure to Nerium indicum leaf extract. This decrease continues till the end of the experiment (96 h) (Figure 1). Analysis of variance indicated that the levels of serum calcium were significantly different between the groups (between intervals F = 9.38, P<0.0001; between treatments F = 55.78, P<0.0001).

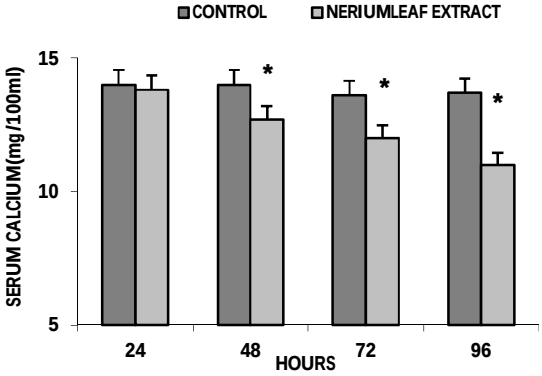

Figure 1. Serum calcium levels of short-term Nerium indicum leaf extract-treated H. fossilis (Values are mean ± S.E. of six specimens.)
Asterisk indicates significant differences (P<0.05) from the control group.

In control fish, two cell types- AF-positive and AF-negative- were noticed after aldehyde fuchsin staining (Figure 2).

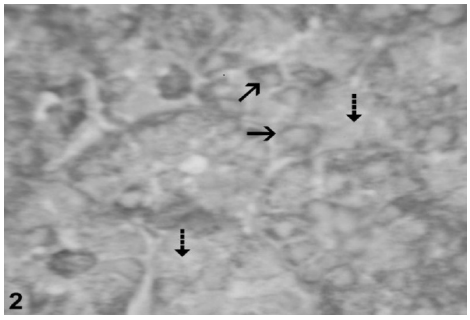

Figure 2. Corpuscles of Stannius of control fish exhibiting AF-positive (arrows) and AF-negative cells (broken arrows); AF x 500.

Corpuscles of Stannius remain unaffected till 72 h in Nerium indicum leaf extract-treated H. fossilis. Increased granulation was seen in the AF-positive cells of CS of fish treated for 96 h (Figure 3). The nuclear volume of these cells exhibited no change throughout the short-term treatment. Slight increase in the nuclear volume of AF-negative cells was recorded after 96 h following Nerium indicum leaf extract (Figure 4). Analysis of variance indicated that in the short-term experiment, the nuclear volume of AF-positive cells was not significant (between time intervals F=0.24, ns; between treatments F= 0.17, ns), whereas for AF-negative cells the values were significant (among time intervals F = 1.00, P <0.403; between treatment F = 1.13, P< 0.294).

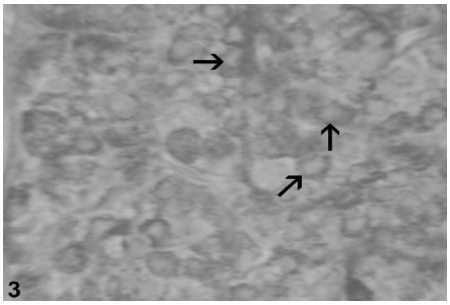

Figure 3. Corpuscles of Stannius of 96 h Nerium indicum leaf extract-treated fish showing increased granulation (arrows) in AF-positive cells; AF x 500.

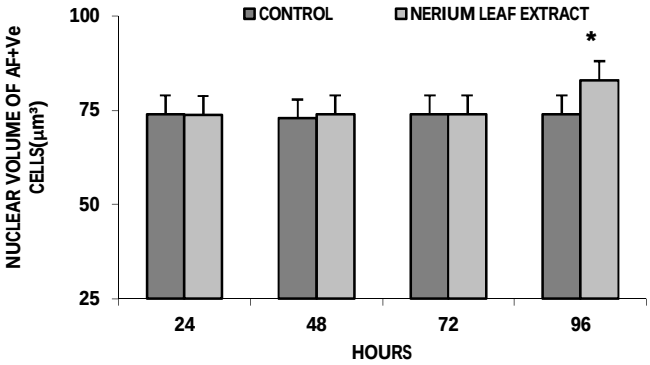

Figure 4. Nuclear volume of AF-negative cells of H. fossilis treated with Nerium indicum leaf extract for short-term.

Each value represents mean ± S.E. of six specimens.
Asterisk indicates significant differences (P<0.05) from the control group.

In the long-term experiment, Nerium indicum leaf extract provoked a decline in the serum calcium levels of H. fossilis on day 14. The levels progressively declined till the close

of the experiment (28 days) (Fig 5). Analysis of variance indicated that the levels of serum calcium for long-term exposure were significantly different between groups (between intervals F = 16.46, P<0.0001; between treatment F = 113.7, P<0.0001).

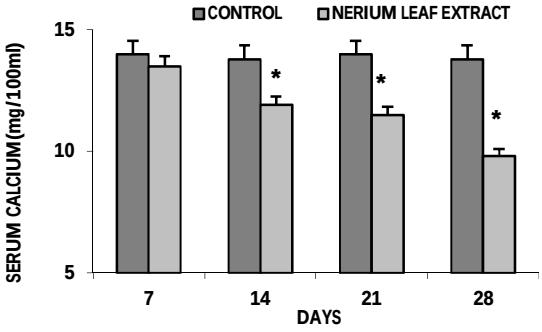

Figure 5. Serum calcium levels of long-term Nerium indicum leaf extract-treated H. fossilis.
Values are mean ± S.E. of six specimens.

Asterisk indicates significant differences (P<0.05) from the control group.

The corpuscles of Stannius of H. fossilis exhibited no alterations in histological structures up to 14 days of exposure to Nerium indicum leaf extract. AF-positive cells of CS depicted increased granulation after 21 days of treatment (Figure 6). The nuclear volume of these cells exhibited a slight decrease on day 21 (Figure 7). A further decrease in the nuclear volume of AF–positive cells of CS has been noticed following 28 days of exposure to Nerium indicum leaf extract (Figure 7). Heavy accumulation of AF-positive granules was observed (Figure 8) and few degenerating cells were visualized at certain places (Figure 9).

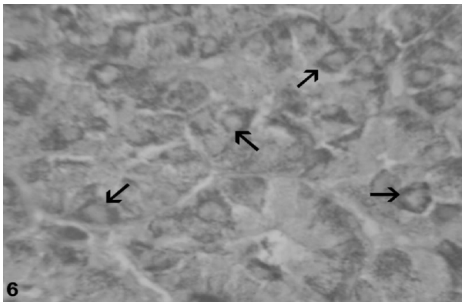

Figure 6. Increased granulation (arrows) in the AF-positive cells of Nerium indicum leaf extract-treated H. fossilis (21days); AF x 500.

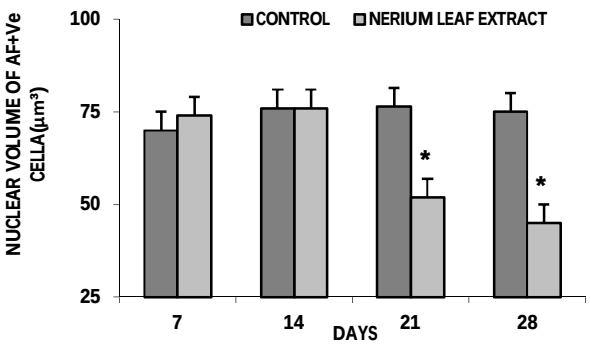

Figure 7. Nuclear volume of AF-positive cells of long-term Nerium indicum leaf extract treated H. fossilis (Each value represents mean ± S.E. of six specimens). Asterisk indicates significant differences (P<0.05) from the control group.

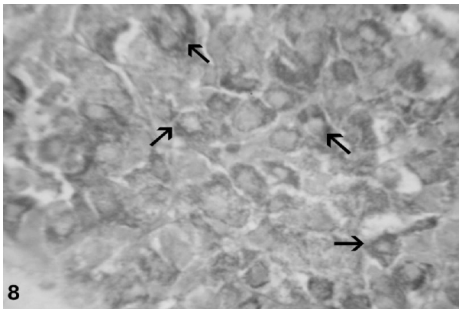

Figure 8. Corpuscles of Stannius of fish exposed to Nerium indicum leaf extract for 28 days showing heavy accumulation of secretary granules (arrows) in AF-positive cells; the degenerating AF-positive cells (broken arrow); AF x 500.

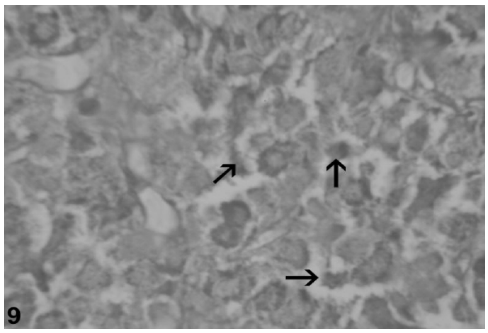

Figure 9. Corpuscles of Stannius of H. fossilis exposed to Nerium indicum leaf extract for 28 days showing degeneration (arrows) in AF-positive cells; AF X 500.

An increase in the nuclear volume of AF-negative cells was noticed after 21 and 28

days following Nerium indicum leaf treatment (Figure 10).

Analysis of variance indicated that in the long-term experiment, the nuclear volumes of AF- positive (between time intervals, F=11.98, P<0.0001; between treatment, F= 36.23, P<0.0001) and AF-negative (between time intervals, F =4.57, P<0.008; between treatment, F =14.45, P<0.0001) cells were significantly different.

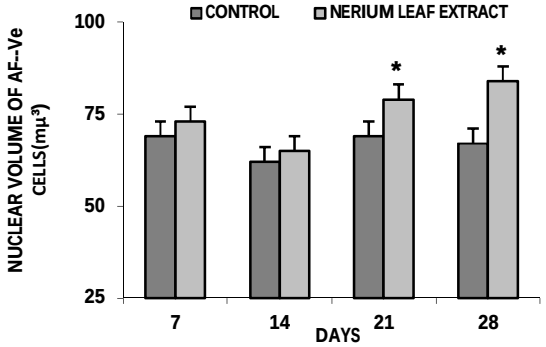

Figure 10. Nuclear volume of AF-negative cells of H. fossilis treated with Nerium indicum leaf extract for long-term (Each value represents mean ± S.E. of six specimens.).
Asterisk indicates significant differences (P<0.05) from the control.

DISCUSSION

In the freshwater catfish, H. fossilis Nerium indicum leaf extract treatment caused hypocalcemia. This is in conformity with the findings of earlier studies that had reported decreased blood calcium content after exposing the fish to various toxicants, such as cypermethrin [24-26], aldrin [27], malachite green [28], cadmium [29-32], propoxur [33], formithion [33], lead [34], and deltamethrin [35-36]. However, no effect had been observed in blood calcium content of DDT-treated flounders Platichthys flesus [37], cadmium-exposed Oncorhynchus niloticus [38], and bifenthrin-treated rainbow trout Oncorhynchus mykiss [39]. Contrary to the findings of the present study, few studies have indicated elevated calcium levels of fish after exposure to pesticides [40-44].

Increased accumulation of secretory granules and a decrease in nuclear volume of AF-positive cells had been noticed in CS of Nerium indicum leaf extract treated fish. Accuulation of secretory granules and decreased nuclear volume of AF-positive cells in response to exposure of fish to toxicants have been recorded earlier by few studies [45-47]. CS has been reported to regulate branchial calcium uptake in the fish through secretion of AF-positive cells (i.e. stanniocalcin, a hypocalcemic hormone) [6, 7, 10, 48-53]. Exposure to Nerium indicum leaf extract caused increased granulation in the AF-positive cells, which can be explained on account of inhibition of the hormonal release and continued biosynthesis of stanniocalcin. The present study derives support from the earlier studies that had also noticed accumulation of AF-positive granules in CS in response to experimentally-induced hypocalcemia in fishes kept in ambient acalcic freshwater [12, 52]. Calcitonin cells of mammals (responsible for the secretion of a hypocalcemic factor-CT) have also been reported to accumulate secretory granules in response to experimentally-induced hypocalcemia [54-58].

CONCLUSION

It can be concluded that Nerium indicum can severely affect the physiology of calcium homeostasis in fishes as alterations in serum calcium content as well as cytological changes in corpuscles of Stannius of the freshwater fish H. fossilis were seen. Calcium is important for several physiological processes, including reproduction. CS is responsible for calcium influx; hence, any alteration in calcium and CS causes physiological disturbances which might severely affect the normal vital functions, growth rate, and survival in nature. Hence, the botanical pesticides should be used with caution near fish inhabiting water reservoirs.

ACKNOWLEDGMENTS

The authors are thankful to research colleagues who have helped conduct the experiment.

REFERENCES

1. Frohne D, Pfänder HJ. A colour atlas of poisonous plants: Wolfe Publishing Ltd.; 1984.

2. Haynes BE, Bessen HA, Wightman WD. Oleander tea: herbal draught of death. Annals of emergency medicine. 1985;14(4):350-3.
3. Blum LM, Rieders F. Oleandrin distribution in a fatality from rectal and oral Nerium oleander extract administration. Journal of analytical toxicology. 1987;11(5):219-21.
4. Goktas O, Mammadov R, Duru ME, Ozen E, Colak AM. Application of extracts from the poisonous plant, Nerium Oleander L, as a wood preservative. African Journal of Biotechnology. 2007;6(17):2000-3.
5. Pang PK. Endocrine control of calcium metabolism in teleosts. American Zoologist. 1973;13(3):775-92.
6. Wendelaar-Bonga SE, Pang PKT. Stannius corpuscles. In: Vertebrate Endocrinlogy: Fundamentals and Biomedical Implications. 1986. p. 436-64.
7. Bonga S, Pang P. Control of Calcium Regulating Hormones in the Vertebrates: Parathyroid Hormone. International Review of Cytology: A Survey of Cell Biology. 1991;128:139-213.
8. Wagner GF. The molecular biology of the corpuscles of Stannius and regulation of Stanniocalcin gene expression. In: Fish Physiology.1994.p.273-306.
9. Fontaine M. Corpuscles de Stannius et regulation ionique (Ca, K, Na) du Milieu interieur de l'Anguille (Anguilla anguilla L.). CR Acad Sci(Paris). 1964;259:875-8.
10. Bonga S. Effect of synthetic salmon calcitonin and low ambient calcium on plasma calcium, ultimobranchial cells, Stannius bodies, and prolactin cells in the teleost Gasterosteus aculeatus. General and comparative endocrinology. 1980;40(1):99-108.
11. Sing S,Srivastav AK. Histophysiology of the corpuscles of stannius of a freshwater catfish, heteropneustes fossilis. Boletim de Physiologie Animale. 1990;14:75-80.
12. Singh S, Srivastav A. Vitamin D3-induced histological changes in the corpuscles of stannius of a freshwater catfish, Heteropneustes fossilis kept either in artificial freshwater, calcium-rich freshwater or calcium-deficient freshwater. Okajimas folia anatomica Japonica. 1996;73(2-3):75-82.
13. Marra L, Groff K, Youson J. The corpuscles of Stannius in arawana (Osteoglossum bicirrhosum), an ancient teleost. Tissue and Cell. 1995;27(4):425-37.
14. Srivastav AK, Srivastava S, Mishra D, Srivastav S, Suzuki N. Effects of deltamethrin on serum calcium and corpuscles of Stannius of freshwater catfish, Heteropneustes fossilis. Toxicological and Environ Chemistry. 2009;91(4):761-72.
15. Swamp K, Ahmad N. Studies of corpuscles of Stannius of Notopterus notopterus in relation to calcium and sodium rich environments. Natl Acad Sci Lett. 1978;1:239-40.
16. Marra LE, Youson JH, Butler DG, Friesen HG, Wagner GF. Stanniocalcin-like immunoreactivity in the corpuscles of Stannius of the bowfin Amia calva L. Cell and tissue research. 1992;267(2):283-90.
17. Ahmad MF, Alim A, Sen N, Lakra G, Mishra K, Raza B, et al. Electron microscopic studies of the corpuscles of Stannius of an airbreathing teleost (Heteropneustes fossilis). Journal of Biosciences. 2002;27(5):509-13.
18. Hasan N, Das S, Srivastav AK, Swarup K. Phosphocalcic response of Stannius corpuscles extract in the freshwater snake, Natrix piscator. Zoological science. 1987;4:391-44.
19. Srivastav AK, Swarup K. Calcemic responses of Stannius corpuscle extract in parrotsPsittacula psittacula. Experientia. 1982;38(7):869-70.
20. Ishibashi K, Imai M. Prospect of a stanniocalcin endocrine/paracrine system in mammals. American Journal of Physiology-Renal Physiology. 2002;282(3):F367-F75.
21. Moore E, Kuestner R, Conklin D, Whitmore T, Downey W, Buddle M, et al. Stanniocalcin 2: characterization of the protein and its localization to human pancreatic alpha cells. Hormone and metabolic research. 1999;31(07):406-14.
22. Trindade D, Silva J, Navarro M, Torriani I, Kobarg J. Low-resolution structural studies of human Stanniocalcin-1. BMC structural biology. 2009;9(1):57.
23. Prasad M, Kumar A, Mishra D, Srivastav S, Suzuki N, Srivastav A. Acute toxicities of diethyl ether and ethanol extracted Nerium indicum leaf to the fish, Heteropneustes fossilis. Nigerian Journal of Natural Products and Medicine. 2009;13(1):53-7.
24. Mishra D, Srivastav S, Srivastav S, Srivastav A. Plasma calcium and inorganic phosphate levels of a freshwater catfish Heteropneustes fossilis in response to cypermethrin treatment. Journal of Ecophysiology and Occupational Health. 2001;1(1):131-8.
25. Mishra D, Rai R, Srivastav SK, Srivastav AK. Histological alterations in the prolactin cells of a teleost, Heteropneustes fossilis, after exposure to cypermethrin. Environmental toxicology. 2011;26(4):359-63.

26. Pandey RK, Malviya A, Das VK. Toxicity of cypermethrin, effects on serum electrolytes (Ca, Mg and Pi) levels and recovery response in fresh water catfish Heteropneustes fossilis Bloch. Int J Biol Chem Sci. 2009;3(5):1182-91.
27. Bano Y. Effects of aldrin on scrum and liver constituents of freshwater catfishClarias batrachus L. Proceedings: Animal Sciences. 1982;91(1):27-32.
28. Srivastava S, Singh N, Srivastava AK, Sinha R. Acute toxicity of malachite green and its effects on certain blood parameters of a catfish, Heteropneustes fossilis. Aquatic Toxicology. 1995;31(3):241-7.
29. Larsson Å, Bengtsson BE, Haux C. Disturbed ion balance in flounder, Platichthys flesus L. exposed to sublethal levels of cadmium. Aquatic Toxicology. 1981;1(1):19-35.
30. Pratap H, Fu H, Lock R, Bonga SW. Effect of waterborne and dietary cadmium on plasma ions of the teleostOreochromis mossambicus in relation to water calcium levels. Archives of environmental contamination and toxicology. 1989;18(4):568-75.
31. Rai R, Srivastav AK. Effects of cadmium on the plasma electrolytes of a freshwater fish Heteropneustes fossilis. Journal of Ecophysiology and Occupational Health. 2003;3(1):63-70.
32. Rai R, Mishra D, Srivastav SK, Srivastav AK. Ultimobranchial gland of a freshwater teleost, Heteropneustes fossilis, in response to cadmium treatment. Environmental toxicology. 2009;24(6):589-93.
33. Singh N, Das V, Srivastava A. Formothion and propoxur induced ionic imbalance and skeletal deformity in a catfish, Heteropneustes fossilis. Journal of Environmental Biology. 1997;18(4):357-63.
34. Rai R, Mishra D, Srivastav SK, Srivastav AK. Short Communication Acute toxicity of cadmium against catfish Heteropneustes Fossilis (Siluriformes: Heteropneustidae) in static renewal bioassays. Ethiopian Journal of Biological Sciences. 2013;7(2):185-91.
35. Srivastav AK, Srivastava S, Srivastav S. Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish, Heteropneustes fossilis. Bulletin of environmental contamination and toxicology. 1997;59(5):841-6.
36. Srivastav AK, Srivastava SK, Mishra D, Srivastav SK. Deltamethrin-induced alterations in serum calcium and prolactin cells of a freshwater teleost, Heteropneustes fossilis. Toxicological and Environ Chemistry. 2010;92(10):1857-64.
37. Haux C, Larsson Å. Effects of DDT on blood plasma electrolytes in the flounder, Platichthys flesus L, in hypotonic brackish water. Ambio. 1979:171-3.
38. Öner M, Atli G, Canli M. Changes in serum biochemical parameters of freshwater fish Oreochromis niloticus following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. Environmental toxicology and chemistry. 2008;27(2):360-6.
39. Velisek J, Svobodova Z, Piackova V. Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (Oncorhynchus mykiss). Veterinarni Medicina. 2009;54(3):131-7.
40. Sastry K, Sharma S. The effect of endrin on the histopathological changes in the liver ofChanna punctatus. Bulletin of environmental contamination and toxicology. 1978;20(1):674-7.
41. Bansal S, Verma S, Gupta A, Dalela R. Physiological dysfunction of the haemopoietic system in a fresh water teleost, Labeo rohita, following chronic chlordane exposure. Part I-Alterations in certain haematological parameters. Bulletin of environmental contamination and toxicology. 1979;22(1):666-73.
42. Dalela R, Rani S, Kumar V, Verma S. In vivo hematological alteration in a freshwater teleost Mystus vittatus following subacute exposure to pesticides and their combination. Journal of Environmental Biology. 1981;2(2):79-86.
43. Sharma M, Goel K, Awasthi A, Tyagi S. Haematological and biochemical characteristics of Heteropneustes fossilis under the stress of Congo Red (diphenyl disazo binaphthionic acid). Toxicology letters. 1982;14(3):237-41.
44. Suzuki N, Tabata MJ, Kambegawa A, Srivastav AK, Shimada A, Takeda H, et al. Tributyltin inhibits osteoblastic activity and disrupts calcium metabolism through an increase in plasma calcium and calcitonin levels in teleosts. Life Sciences. 2006;78(21):2533-41.
45. Srivastav AK, Srivastava SK, Tripathi S, Mishra D. Chlorpyrifos-based commercial formulation: alterations in corpuscles of Stannius of catfish. International Journal of Environment and Health. 2010;4(4):323-32.
46. Mishra D, Srivastav S, Suzuki N, Srivastav A. Corpuscles of Stannius of a freshwater teleost,

Heteropneustes fossilis in response to metacid-50 treatment. Journal of Applied Science and Environmental Management. 2009;13:69-77.
47. Mishra D, Tripathi S, Srivastav SK, Suzuki N, Srivastav AK. Corpuscles of Stannius of a teleost, Heteropneustes fossilis following intoxication with a pyrethroids (cypermethrin). North-Western Journal of Zoology. 2010;6(2):203-8.
48. Meats M, Ingleton P, Jones IC, Garland H, Kenyon C. Fine structure of the corpuscles of stannius of the trout, Salmo gairdneri: Structural changes in response to increased environmental salinity and calcium ions. General and comparative endocrinology. 1978;36(4):451-61.
49. Pang PK, Pang RK. Hormones and calcium regulation in Fundulus heteroclitus. American Zoologist. 1986;26(1):225-34.
50. Srivastav AK, Srivastav SP. Corpuscles of Stannius of Clarias batrachus in response to 1, 25 dihydroxyvitamin D3 administration. Zoological science. 1988;5(1):p197-200.
51. Singh S. Studies of endocrine glands regulating calcium and inorganic phosphorus homeostasis in Heteropneustes fossilis. [PhD Thesis]. University of Gorakhpur,India, 1990.
52. Tiwari PR. Endocrinal regulation of calcium in teleost. [PhD Thesis]. University of Gorakhpur,India. 1993.
53. Tseng D-Y, Chou M-Y, Tseng Y-C, Hsiao C-D, Huang C-J, Kaneko T, et al. Effects of stanniocalcin 1 on calcium uptake in zebrafish (Danio rerio) embryo. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2009;296(3):R549-R57.
54. Gittes RF, Toverud SU, Cooper CW. Effects of hypercalcemia and hypocalcemia on the thyrocalcitonin content of rat thyroid glands. Endocrinology. 1968;82(1):83-90.
55. Leitzb H, Donath K. Cytochemical evidence for the presence of hormonal peptides in thyroid C cells. In: Calcitonin 1969. 1970.p. 227-39.
56. Biddulph DM, Maibenco HC. Response of hamster thyroid light cells to plasma calcium. The Anatomical Record. 1972;173(1):25-43.
57. Swarup K, Tewari N, Srivastav AK. Response of calcitonin cells, parathyroid glands and bone to prolonged calcitonin administration in the Indian palm squirrel, Funambulus pennanti (Wroughton). Cells Tissues Organs. 1980;106(2):180-91.
58. Srivastav AK, Swarup K. Effect of calcitonin on calcitonin cells, parathyroid glands and serum electrolytes in the house shrew (Suncus murinus). Cells Tissues Organs. 1982;114(1):81-7 .